VOLUME XXXI OCTOBER, 1952 NUMBER 10

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

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The Journal of Clinical Investigation

Printed by the Lancaster Press, Inc., Prince and Lemon Sts., Lancaster, Pa. Published by the American Society for Clinical Investigation. Publication Office, c/o Presbyterian Hospital, 622 West 168th St., New York 32, N. Y.

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Acceptance for mailing at special rate of postage provided for in paragraph (d-2), section 34.40, P.L. & R. of 1948, authorized February 13, 1950

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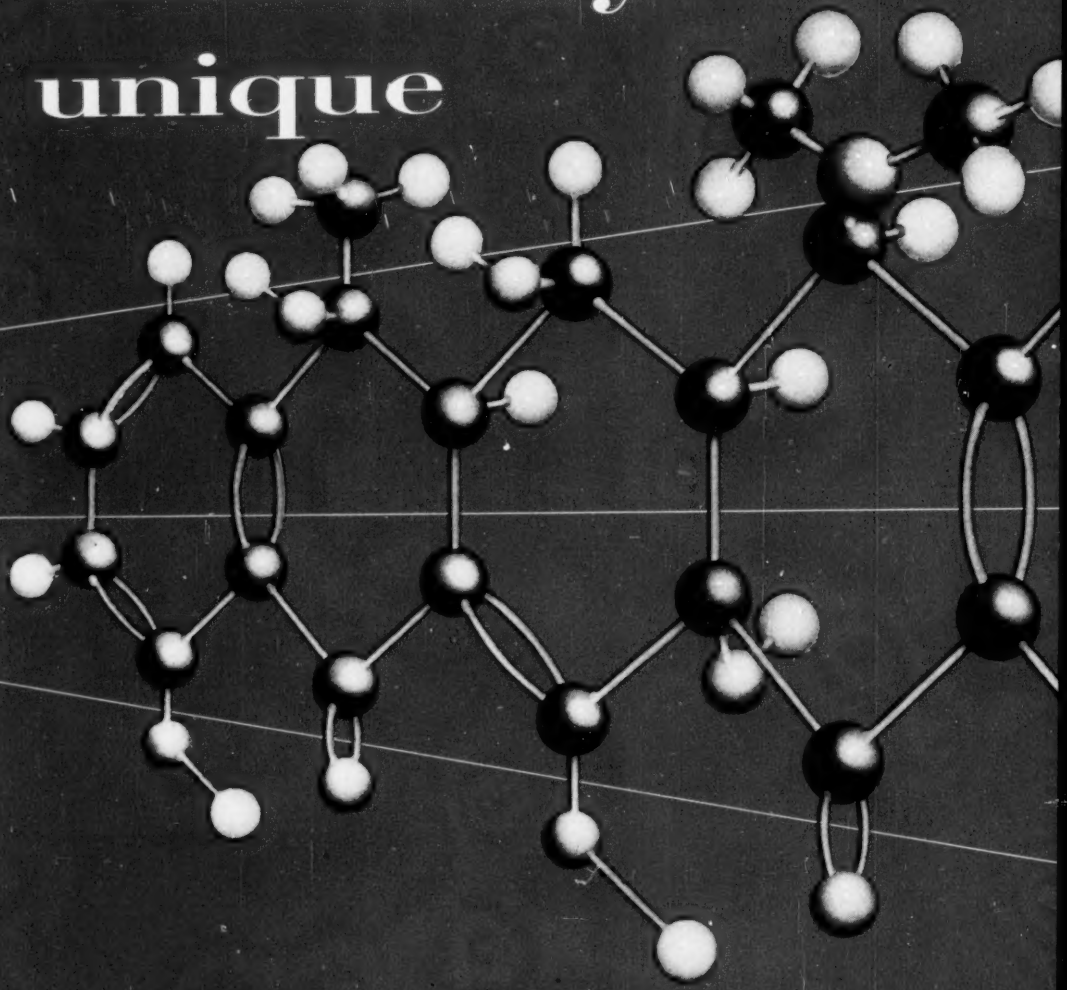

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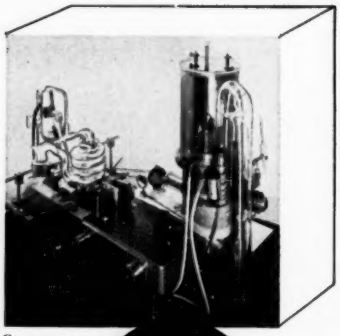

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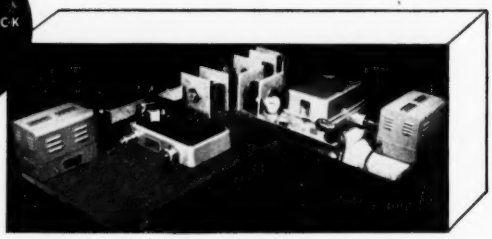

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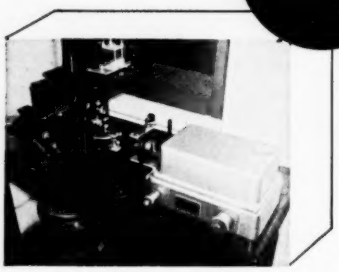

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A STUDY OF THE NUTRITIONAL DEFECT IN WERNICKE'S SYNDROME

THE EFFECT OF A PURIFIED DIET, THIAMINE, AND OTHER VITAMINS ON THE CLINICAL MANIFESTATIONS[1]

By GERALD B. PHILLIPS, MAURICE VICTOR, RAYMOND D. ADAMS, AND CHARLES S. DAVIDSON

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(Submitted for publication December 15, 1951; accepted July 29, 1952)

The salient clinical features of Wernicke's syndrome (1) are partial to complete paralysis of extra-ocular muscles (most commonly the external recti), nystagmus, ataxia and mental disturbances. Postmortem examination reveals changes in the nervous structures adjacent to the third and fourth ventricles and the aqueduct. The lesions are characterized by varying degrees of necrosis of both nerve cells and nerve fibers with appropriate reactions of microglia and astrocytes, alteration of the small blood vessels, and in some cases, petechial hemorrhages (2–5).

The syndrome is usually associated with chronic alcoholism and for that reason has sometimes been attributed to a neurotoxic effect of the alcohol; but the occurrence of the same clinical sequence in non-alcoholic patients with malnutrition (1, 2, 4–8) has directed attention to nutritional deficiency as the etiological basis. Moreover, similar pathological lesions have been produced in the rat (9, 10), fox (11, 12), and pigeon (3, 9, 13, 14), by maintaining these animals on thiamine-deficient diets.

The first extensive clinical investigation of the nutritional disorder of Wernicke's syndrome was conducted by Jolliffe, Wortis, and Fein (15), who followed twenty-seven patients on various diets with specific vitamin supplements and concluded that the ophthalmoplegia responded to thiamine but that the whole syndrome was probably due to a "combination of several nutritional deficiencies." Other reports concur that thiamine deficiency plays a definite role in the etiology of this syndrome (7, 8, 16).

The study reported in the present communication was designed to define more clearly the effect of bed rest, withdrawal of alcohol, and administration of vitamins on the individual clinical signs comprising Wernicke's syndrome. Patients with typical manifestations of this syndrome were maintained for varying periods of time on a purified diet consisting solely of glucose and minerals. At appropriate times specific vitamins were added. The principal components of the clinical picture, *i.e.*, the ophthalmoplegia, nystagmus, ataxia, and mental disturbances, were examined at frequent intervals.

MATERIALS AND METHODS

Nine patients with the typical signs of Wernicke's syndrome were observed on the wards of the Boston City Hospital. Only patients with a definite degree of ophthalmoplegia who had received no food after admission were included. All patients were men, white, and over 40 years of age. Dietary histories could not be obtained from eight because of mental disturbances, but it was learned from their families and friends that they were confirmed chronic alcoholics and had been drinking heavily and eating little or no food in the weeks preceding entry to the hospital. Objective evidence of undernutrition, such as looseness of the skin and thinness of muscle, was observed in all cases. In addition, six (Cases Nos. 1 through 6) had liver disease as indicated by abnormal "liver function" tests, and in three (Cases Nos. 1, 3, and 4) this was confirmed by needle biopsy. No evidence of beriberi was found in any patient except for a frank peripheral neuropathy in one (Case No. 6). Pellagrinous skin lesions were seen in only one patient (Case No. 9), whereas two showed tender, red tongues (Cases Nos. 2 and 3). One patient (Case No. 9) had perifollicular hemorrhages and another (Case No. 2) manifested hoarseness. No other signs suggesting deficiency disease were

[1] The expenses of this investigation were defrayed in part by grants from Merck and Company, Inc., Rahway, New Jersey, and Sharpe and Dohme, Inc., Glenolden, Pennsylvania.

detected, and in no case was any other serious abnormality found, except for delirium tremens, which was evident in Case No. 1. Significant fever was not present in any patient. All the patients survived their disease.

The subjects received no food from the time they entered the hospital and all, except Case No. 9, were started immediately on a regimen consisting of the oral administration of a solution which contained 200 gm. of glucose and 1.3 gm. of sodium chloride per liter of water. Case No. 9 was given an infusion containing multiple vitamins [2] including thiamine on admission and was started on a regular hospital diet twelve hours later. Cases Nos. 3, 5, and 6 received potassium chloride in addition to glucose and saline in an attempt to eliminate potassium deficiency as an etiological component of the mental disturbance. Occasionally, prophylactic penicillin and small doses of paraldehyde were given. No other form of sustenance or medication was administered except for certain vitamins as specified below. An exact measure of the amount of the solution consumed by each patient was difficult to obtain, but each ingested approximately 1 to 2 liters a day. After 3 to 11 days of the purified diet, a regular hospital diet was instituted for the remainder of the hospitalization. The period of observation on the purified diet was not extended beyond 11 days because of the serious nature of Wernicke's syndrome. Three subjects vomited infrequently, but it was impossible to determine whether this was due to an alcoholic gastritis, to Wernicke's syndrome, to the possible gastric irritation of glucose and saline, or to some other factor.

All the patients, except Case No. 4, were confined to bed until they were able to walk without assistance. Case No. 6 could not walk during his hospitalization but was allowed to sit in a chair after the third week in the hospital.

The ophthalmoplegia, nystagmus, ataxia, and mental disturbances were observed closely and an attempt was made to quantitate them on a 1 + to 4 + scale. Since the principal ocular derangement in all the cases was a bilateral weakness of the external rectus muscles, the power of abduction of the eyes was used as an index of the degree of ophthalmoplegia. A total inability to abduct the eye beyond the midline was considered as 4 +, a slight abductive movement as 3 +, moderate abduction as 2 + and almost full lateral movement with only a small amount of sclera showing between the limbus and outer canthus or an inability to sustain lateral gaze for more than a few seconds, as 1 +. Coarse, well-sustained nystagmus demonstrated on lateral or vertical gaze was designated as 4 +, lesser degrees of it as 3 + or 2 + and a barely perceptible nystagmus as 1 +. With respect to ataxia, 4 + denoted an inability to walk even with some assistance; 3 + meant that the patient could walk if aided; 2 + indicated ability to walk alone but with staggering; and 1 + represented competent locomotion but with slight ataxia. The degree of psychic derangement was most difficult to evaluate. In these patients several aspects of the mental disturbance were discernible, i.e. alertness and the capacity for sustained mental activity, confusion, and memory defect with confabulation. None of the patients exhibited an appreciable variation in the degree of amnesia during the period of observation. Rough quantitative data are given only on the confusional aspect. Four plus implied extreme confusion, often with such total disorientation that the patient did not know whether he was standing or lying, dressed or undressed, indoors or outdoors, or whether it was day or night. He failed to recognize and often misidentified objects and people around him. He could not carry on a conversation or answer questions adequately. Three plus represented the state in which there was greater awareness of the immediate environment, but still gross disorientation as to place and time. Two plus and one plus indicated lesser degrees of confusion, particularly in regard to orientation in place and time. Often confabulation was more evident as the patient improved. None of the patients were disoriented as to person.

[2] "Berocca C" (Hoffmann-LaRoche). Each 2 cc. contains thiamine hydrochloride, 10 mgm.; riboflavin, 6 mgm.; niacinamide, 80 mgm.; pyridoxine hydrochloride, 6 mgm.; panthenol (equivalent to 6 mgm. Ca pantothenate), 6 mgm.; ascorbic acid, 100 mgm. with 3% gentisic acid ethanolamide as a preservative.

RESULTS

Since the regimen varied somewhat from patient to patient, the clinical data on each will be considered separately.

Case 1 (Table I). This 67-year old man was admitted to the hospital after collapsing in court. His son testified that he was accustomed to drinking in long sprees, the one preceding his admission having lasted 2 months. During this time his diet was said to have consisted of a sandwich at long and irregularly spaced intervals.

He was, on first examination, slightly tremulous and florid. There was considerable loss of muscle. The liver, which was tender and palpable 4 cm. below the right costal margin, showed a large amount of fat and minimal, if any, fibrosis on biopsy; in addition, "liver function" tests were abnormal.

Questions were answered in a normally alert and cooperative manner. He was disoriented in place and time. In addition, he did not know what clothing he had on; he spoke of lying on a bench in the station waiting to be picked up by his daughter. He did not realize that he was ill and gave quite irrational answers, the responses varying from one minute to the next.

Since he had a coarse tremor and transient hallucinations, he was thought to have delirium tremens in addition to his Wernicke's syndrome.

TABLE I

Case No. 1—B. R., male, age 67

Hospital day	1	2	3	4	5*	6	7	8*	9*	10	11*	12	15	19	35
External rectus paralysis	3+	3+	3+	3+	4+	1+	±	±	0	0	0	0	0	0	0
Horizontal nystagmus	3+	3+	3+	3+	3+	3+	3+	2+	2+	1+	1+	1+	1+	1+	1+
Ataxia	4+	4+	4+	4+	4+			3+	2+	2+	2+	2+		1+	1+
Confusion	4+	4+	3+	3+	3+	2+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Therapy	Glucose and saline →				Thiamine 100 mgm. i.m.			Thiamine 200 mgm. i.m. daily →			Regular hospital diet →				
								Niacin 1000 mgm. p.o. daily →							
								Multiple vitamins† →							

* Measurements recorded on the day a therapy was started are pre-therapy.
† "Berocca C" (see footnote 2) 4 cc. i.v. daily.

There was a bilateral internal strabismus due to an almost complete external rectus paralysis, as well as a coarse horizontal nystagmus on attempted lateral gaze. His actions betrayed a marked diplopia. Ataxia was manifest on standing; he could not walk even with assistance.

He was given only glucose and saline for four days, during which time his external rectus paralysis became even worse, the nystagmus and ataxia remained the same, his hallucinations and tremor disappeared, and he seemed somewhat less confused. On the fifth day he was started on 100 mgm. of thiamine intramuscularly twice a day in addition to the glucose and saline. Within 5 hours after the first dose, his external rectus paralysis was barely detectable. After three days of thiamine (Day 8), external rectus weakness was only questionably present, nystagmus was less, and walking was accomplished with assistance, but his mental state, which seemed to have temporarily improved within 24 hours after thiamine was instituted, was no better than before thiamine. On Day 8, 1000 mgm. of niacin orally per day was added. On Day 9, no external rectus weakness could be detected, and he walked without assistance for the first time; the mental status, however, showed no change. On this day, a multiple vitamin preparation,[3] given intravenously, was added to the regimen. On Day 10, nystagmus was slightly less and ataxia and mental status were the same as on the previous day. Food was started on Day 11. Thirty-five days after entry, the patient had no ocular palsies, minimal horizontal nystagmus, and a somewhat wide-based gait. His mental disorganization was as marked as prior to the institution of thiamine. Confabulation was more spontaneous at this time than on entry.

Case 2 (*Table II*). This 57-year old single man was discovered in his room in an irrational and neglected state. He had been there for an indeterminate period of time and was quite unable to supply a history of his illness.

He was very thin and unkempt. His liver was enlarged to two finger-breadths below the right costal margin, and there was laboratory evidence of abnormal function. He was alert, but inattentive and incapable of sustaining a conversation. His remarks and behavior were facetious and inappropriate. He had no idea of the place or date and could give no rational answers to any questions concerning his past history or circumstances of his arrival to the hospital. There was diplopia in all directions, and he usually kept one eye closed.

[3] "Berocca C"; 4 cc. were given daily.

TABLE II

Case No. 2—E. H., male, age 57

Hospital day	1	2	3	4	5*	6	7	8*	9	10*	18
External rectus paralysis	3+	3+	4+	4+	4+	1+	1+	0	0		0
Horizontal nystagmus	0	0	0	0	0	3+	3+	2+	1+		1+
Ataxia	3+	3+	3+	3+	3+	2+	2+	1+	1+		1+
Confusion	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Therapy	Glucose and saline →				Thiamine 100 mgm. i.m.	200 mgm. s.c. daily →				Regular hospital diet →	
								Multiple vitamins† →			

* See footnote to Table I.
† "Berocca C" (see footnote 2) 4 cc. i.m. daily.

Abduction of each eye was limited to a few millimeters, and nystagmus was not evident. He needed considerable coaxing and support to stand or take a few steps, both of which acts were carried out in a markedly ataxic manner. Ankle jerks were absent and knee jerks depressed, but there were no other signs of neuropathy.

After four days of the glucose and saline regimen, the paralysis of his external recti was complete. Nystagmus was absent and the ataxia remained unchanged. His responses were even less adequate than on entry and he was more careless of his speech and dress. One hundred mgm. of thiamine were then injected intramuscularly and four hours later, there having been no improvement, another 100 mgm. were given. Within a half hour after the second dose, strabismus and diplopia had disappeared, external rectus motion was almost normal, and a coarse horizontal nystagmus became evident for the first time. After three days of thiamine administration, external rectus paralysis was gone, nystagmus was less and ataxia was much improved; his confusional state, however, remained unchanged. A multiple vitamin preparation [3] was given intramuscularly for the next two days without discernible improvement in the mental state. On Day 10 food was started. Eighteen days after admission, his ocular disturbance was limited to a fine horizontal nystagmus on far lateral gaze, and his gait was slightly wide-based and unsteady. He was more alert, entered into sustained conversation more readily, and required no stimulation to confabulate. His confusion, however, was as marked as prior to the administration of thiamine and other vitamins.

Case 3 (Table III). This 46-year old man, who had been hospitalized nine months previously for cirrhosis of the liver, was readmitted because of irrational behavior and inability to walk for three to four days.

Since youth he had been consuming on the average of 2 qts. of wine daily. His family stated that for at least three years he had eaten only one meal daily, and then "only picked at his food."

There was general emaciation, roughness and dryness of the skin, and enlargement of the liver to 10 cm. below the right costal margin. "Liver function" tests were deranged, and a liver biopsy revealed a large amount of fat with definite fibrosis.

The patient was alert and not tremulous or hallucinated. He realized he was in bed, but literally nothing more. His conversation showed a total lack of understanding of what was going on around him and he misidentified the examiner and other patients. Disorientation in place and time was severe. In addition, there was a pronounced memory defect from the time of entry, and the tendency to confabulate became obvious within a few days.

TABLE III

Case No. 3—P. F., male, age 46

Hospital day	1	2	3*	4	5	6	7	8	9*	20
External rectus paralysis	2+	2+	4+†	±	±	0	0	0	0	0
Horizontal nystagmus	1+	1+	0	1+	1+	1+	1+	1+	0	0
Vertical nystagmus	0	0	0	1+	1+	0	0	0	0	0
Ataxia	3+	3+	3+	2+	2+	2+	2+	2+	1+	1+
Confusion	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Therapy	Glucose and saline →		Thiamine 5 mgm. i.v.	15 mgm. p.o. daily →					Regular hospital diet →	

* See footnote to Table I.
† Paralysis of lateral gaze.

On entry there was a mild internal strabismus; abduction of both eyes was incomplete and a mild horizontal nystagmus developed on attempting this movement. The pupils were normal in size and reacted briskly to light. His gait was ataxic, and he required support to walk. Knee and ankle jerks were absent, but motor power and sensation were adequate.

On the third day of the glucose and saline regimen, there was an increase of the ocular defect manifested by a paralysis of gaze; there was almost a complete inability to perform lateral conjugate movements of the eyes although upward and downward gaze was unimpaired. Moreover, the pupils became miotic and non-reacting. Five milligrams of thiamine were then administered intravenously. Within six hours, lateral gaze was almost normal, and the pupils became dilated and reactive to light. After five subsequent days of thiamine (15 mgm. orally per day) in addition to the glucose and saline, external rectus paralysis and nystagmus were not detectable, and the ataxia was only slight in degree. The mental disturbance, however, was unimproved. After 11 subsequent days of food, ataxia was evident only on heel-to-toe walking, and there was still no change in mental status.

Case 4 (*Table IV*). This 43-year old man was admitted because of advanced liver disease and complaints of double vision. He gave a history of alcoholic excess (50 to 60 glasses of ale daily) for 20 years. In the last three months anorexia was extreme, and he claimed to have lost 25 pounds in weight in the month preceding entry into the hospital. Diplopia was present for seven days before admission. On physical examination, his liver was found to be enlarged to three to four finger-breadths below the right costal margin; "liver function" tests were markedly altered and a liver biopsy revealed a marked degree of fat and fibrosis.

When first examined, no mental defect was demonstrable. The abnormal eye signs consisted of an alternating internal strabismus, a moderate bilateral external rectus weakness, and a coarse horizontal nystagmus on lateral gaze. Ataxia was slight and difficult to assess because of an old right peroneal palsy.

On the second day of the glucose and saline diet he developed a quiet confusion, which increased over the subsequent two days to the point where he was wandering aimlessly around the ward, disoriented, irrational, and unaware of the meaning of all that was happening around him. The ocular disorder and ataxia remained unchanged. On Day 4 he was given 500 mgm. of niacin orally; 24 hours later (Day 5) his condition was no different except for an increase in the external rectus weakness and a decrease in horizontal nystagmus. On Day 5 he was given 50 mgm. of thiamine intravenously and in one and a quarter hours there was a definite decrease in external rectus palsy; in one

TABLE IV

Case No. 4—P. S., Male, age 43

Hospital day	1	2	3	4*	5*	6*	7	8	9	10	11	14
External rectus paralysis	2+	2+	2+	2+	3+	0	0	0	0	0	0	0
Horizontal nystagmus	3+	3+	3+	3+	2+	2+	2+	1+			1+	±
Ataxia	1+	1+	1+	1+	1+	1+	1+	1+				0
Confusion	0	1+	2+	3+	3+	3+	0	2+	2+	0		0
Therapy	Glucose and saline →			Niacin 500 mgm. p.o.	Thiamine 50 mgm. i.v. Niacin 1000 mgm. p.o. Multiple vitamins†	Regular hospital diet →						

* See footnote to Table I.
† See footnote to Table I.

and three-quarter hours he could abduct his eyes almost to their full extent, and in three hours he had full range of movement, but still had difficulty in sustaining lateral gaze. Several hours later he was given 1000 mgm. of niacin orally and a multiple vitamin preparation [3] intravenously. On Day 6, 48 hours after starting niacin and 24 hours after thiamine and multiple vitamins, he was still thoroughly confused even though no external rectus weakness was evident and nystagmus was less. The regular hospital diet was begun that evening and when seen the next morning (12 hours later) he showed no mental abnormality, but still had nystagmus and slight ataxia. One day later, however, he lapsed into a confused state, which reversed completely within 36 hours. On the fourteenth day after admission he had only a questionable nystagmus, but no abnormality of ocular movement, gait, or mental function.

Case 5 (Table V). This 58-year old man

TABLE V

Case No. 5—C. Z., male, age 58

Hospital day	1*	2*	3	4*	5	6	7	8	9	10	11	23
External rectus paralysis	3+	4+	1+	±	±	±	±	±	±	±	0	0
Horizontal nystagmus	2+	1+	3+	3+	3+	3+	3+	3+	2+	2+	1+	0
Vertical nystagmus	4+	4+	±	0	0							
Ataxia	4+	4+	3+	3+	3+	2+	2+	2+	2+	2+	2+	1+
Confusion	3+	3+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
Therapy	Glucose and saline → Multiple vitamins§ → Thiamine 50 mgm. i.v.			Regular hospital diet →								

* See footnote to Table I.
§ The following vitamins were given orally daily: Ca pantothenate, 100 mgm.; pyridoxine, 100 mgm.; folic acid, 10 mgm.; ascorbic acid, 250 mgm.; riboflavin, 10 mgm.; niacin, 250 mgm. In addition, 10 μgm. of vitamin B_{12} were administered intramuscularly on Day 1.

was brought to the hospital by the police because of the acute onset of confusion and abdominal distress. He had been leading a derelict's existence for many years, moving from one job and lodging house to another and drinking steadily. In the months preceding his admission, his family had noted an aversion to food and a severe weight loss of undetermined amount.

He was a wasted, untidy man with evidence of mild liver disease. He was alert and cheerful and not hallucinating. Orientation in place and time, recent and past memory, judgment and insight were all impaired. His ocular defect consisted of internal strabismus, almost complete paralysis of the external recti, and a marked vertical and fine horizontal nystagmus. He exhibited severe ataxia, despite the presence of normal reflexes, motor power and sensation.

He was given glucose and saline and multiple vitamins (see Table V). Twenty-four hours later his condition was the same except that his external rectus paralysis had become complete and horizontal nystagmus less. The vitamins of the previous day were repeated, except for vitamin B_{12}, with no further change. Later in the day he was given 50 mgm. of thiamine intravenously and within four hours he could abduct his eyes almost completely. Associated with this improvement was an increase in horizontal nystagmus. On Day 3, only slight external rectus weakness was evident and although the horizontal nystagmus was still rather marked, the presence of any vertical nystagmus was doubtful. Ataxia was somewhat less and his mental status was improved to the extent that he was now oriented in place; also, confabulation was now becoming prominent.

On Day 4, food was started, and 19 days later no ocular abnormality of any kind was elicitable, and ataxia was slight in degree. He was oriented in place but grossly disoriented in time; no improvement in retentive memory had occurred, but confabulation was more easily provoked than on admission.

Case 6 (*Table VI*). This 50-year old man was admitted because of irrationality and inability to walk of two days duration. On physical examination, his liver was found to be three finger-breadths below the right costal margin. In addition, there were slight abnormalities of the "liver function" tests.

On entry he was profoundly confused in place and time as well as to his immediate bodily environment. He had no idea what clothing he had on or who the examiner was. His memory was equally badly disordered, and confabulation was detectable at the first examination. He showed a moderate external rectus weakness, slight horizontal nystagmus and diplopia. Standing was impossible because of a severe peripheral neuropathy, and thus ataxia could not be assessed.

During the first four days of glucose and saline his nystagmus and confusion grew worse, and

TABLE VI

Case No. 6||*—J. M., male, age 50*

Hospital day	1	2	3	4	5*	6	7*	8	9	10	23	35
External rectus paralysis	2+	2+	2+	2+	2+	3+	4+‡	1+	1+	1+	0	0
Horizontal nystagmus	1+	1+	1+	2+	2+	0	0	3+	3+		3+	3+
Confusion	3+	3+	3+	4+	4+	4+	4+	3+	3+	3+	3+	3+
Therapy	Glucose and saline →				Multiple vitamins§		Thiamine 100 mgm. i.v.	Regular hospital diet¶ →				

* See footnote to Table I.

‡ Paralysis of gaze.

§ The following vitamins were given orally daily: Ca pantothenate, 100 mgm.; pyridoxine, 100 mgm.; folic acid, 10 mgm.; ascorbic acid, 500 mgm.; riboflavin, 10 mgm.; niacin, 500 mgm. In addition, 10 μgm. of vitamin B_{12} were administered intramuscularly on Day 5.

|| No evaluation of ataxia was possible because of peripheral neuropathy.

¶ Started evening of Day 7.

TABLE VII

Case No. 7—F. L., male, age 60

Hospital day	1*	2	3	4	5	6	10	11*	12	15	18	29
External rectus paralysis	3+	1+	1+	1+	1+	1+	1+	0	0	0	0	0
Horizontal nystagmus	0	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Vertical nystagmus	2+	2+	2+	2+	2+	0	0	0	0	0	0	0
Ataxia	4+	—	—	—	3+	2+	2+	2+	2+	1+	1+	1+
Confusion	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Therapy	Glucose and saline → Thiamine 100 mgm. i.m. daily →							Regular hospital diet →				

* See footnote to Table I.

there was an increase in general weakness. On Day 5, he was given several vitamins exclusive of thiamine (see Table VI). On Day 6, there was almost complete external rectus paralysis with a concomitant disappearance of nystagmus. He was noisy, irrational, uncooperative and generally weaker. By Day 7, he had lost all movements of his eyes except for upward gaze, and a vertical nystagmus was noted for the first time. One hundred milligrams of thiamine were then injected intravenously and within 2 hours lateral conjugate movements improved considerably and horizontal nystagmus became prominent. A few hours later food was started. On Day 8, his external rectus weakness was slight and he was generally more alert and cooperative and, perhaps, less confused. Thirty-five days after admission, examination disclosed absence of external rectus weakness, a fine vertical nystagmus, and a coarse horizontal nystagmus. His mental status, except for increased alertness and attentiveness, was unchanged. The neuropathy was still very severe.

Case 7 (Table VII). This 60-year old man collapsed in court while answering charges of drunkenness. He showed generalized wasting but no definite evidence of liver disease. He lay quietly in bed and would reply only to persistent questioning, being quite content to turn over and suspend the conversation. He could give only his name correctly. He thought he was in a schoolhouse, mistook the examiner for a saleswoman, and had no idea of the date, his address, or the meaning of what he saw around him. His eyes could be abducted only slightly from the mid-line, and no horizontal nystagmus was demonstrable. Upward gaze was limited, and a vertical nystagmus developed on this movement. He was unable to stand without help and reeled when attempting to walk. Motor power was intact, but knee and ankle jerks could not be obtained.

After the initial examination, the diet of glucose and saline was instituted and continued for ten days. He was given, on entry and once daily thereafter, 100 mgm. of thiamine intramuscularly as the only supplement.

Three hours after the first injection of thiamine, ocular movement was improved, and a coarse horizontal nystagmus appeared; 12 hours later abduction was almost complete. Over the next nine days there was gradual improvement in his ability to sustain lateral gaze. Vertical nystagmus disappeared by the sixth day, but the horizontal nystagmus remained unchanged from the time it appeared. The ataxia showed slow but progressive improvement over the ten-day period; at the end of this time he was getting about freely, although his gait was still wide-based, and he staggered slightly on turning quickly. Mentally the patient became more alert and cheerful. He entered into conversation readily and confabulated freely. Only in these respects was there a change in his mental status since admission. Food was started on the eleventh day. Eighteen days later ataxia was further improved but no essential change could be detected in his horizontal nystagmus or confusion.

Case 8 (*Table VIII*). This 74-year old man was brought to the hospital by the police after having collapsed in a doorway. He was poorly nourished and his skin was dry, loose, and covered with pediculous excoriations. No definite evidence of liver disease was detected. He spent most of the day lying in bed with his eyes closed, a state from which he could be aroused easily. He then answered questions alertly, although he did not enter into spontaneous conversation. He was consistently and grossly disoriented in time, being wrong as to the season, and giving the year as 1970. His confusion in regard to his surroundings varied. At times he thought he was in a hospital, at others in a hotel. Memory span was impaired to the extent that he could not recall two facts after a lapse of one minute. Confabulation could be elicited at the first examination. There was marked bilateral external rectus weakness, and a coarse horizontal nystagmus developed on lateral gaze. The patient walked very slowly, with small uncertain steps, requiring support occasionally, especially on turning. There were no signs of peripheral neuropathy.

After an observation period of 24 hours, the glucose and saline diet was instituted and continued for 11 days, with a daily supplement of 100 mgm. of thiamine intramuscularly for the first ten days. The patient was not seen again until 12 hours after the first dose of thiamine, at which time there was a marked improvement in the external rectus palsy; complete abduction, however, was accomplished only after five days of therapy. The nystagmus also was reduced in degree within 12 hours; after three days it was barely detectable on left lateral gaze only, a state which then persisted for as long as the patient was followed. His ataxia improved progressively over the ten days, although it was still evident at the end of this time.

By the end of the ten-day period of the purified diet and thiamine administration, there was a slight but definite improvement in mental state. He was less confused in time, consistently giving the correct month and approximating the year. When questioned on general information as well as events in his own life, he gave many more correct answers than on admission. His memory span was increased by several minutes; confabulation was unchanged.

A multiple vitamin preparation [8] was given intramuscularly on Day 12 with no further change in the patient's condition. One day later food was started and after four days of the regular hospital diet, there was still no discernible change.

Case 9. This 68-year old man was admitted after collapsing in the street. He showed signs of general neglect and marked wasting. There were numerous petechiae over his thighs and knees suggesting scurvy. No evidence of liver disease was found. He was disinclined to speak, but his few responses indicated profound disorientation and memory loss. His eyes showed an almost complete external rectus paralysis, coarse horizontal nystagmus, and fine vertical nystagmus. His gait was broad-based and his steps short and uncertain

TABLE VIII

Case No. 8—D. S., male, age 74

Hospital day	1	2*	3	4	5	6	7	8	9	10	11	12*	13*	17
External rectus paralysis	3+	3+	1+	1+	1+	1+	0	0	0	0	0	0	0	0
Horizontal nystagmus	3+	3+	2+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
Ataxia	3+	3+	3+	2+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
Confusion	3+	3+	3+	3+	3+	3+	3+	2+	2+	2+	2+	2+	2+	2+
Therapy		Glucose and saline → Thiamine 100 mgm. i.m. daily →										Multiple vitamins†	Regular hospital diet →	

* See footnote to Table I.
† See footnote to Table II.

in the face of adequate motor power, normal reflexes, and intact sensory function.

On the night of admission he was given 500 mgm. of thiamine and a multiple vitamin preparation [4] intravenously. When he was next seen twelve hours later, his external rectus weakness was strikingly improved and consisted only of an inability to sustain lateral gaze. Otherwise, his condition was essentially unchanged. Food was started at this time. Four days later no external rectus weakness was detectable, but nystagmus and ataxia were the same; the patient was much more alert and cheerful, and he could be induced to confabulate. Re-evaluation 40 days after admission disclosed no oculomotor weakness and only slight horizontal and vertical nystagmus. Ataxia was present only on attempting to walk a straight line. Memory for recent events was virtually nil, and he confabulated freely. In other respects his mental derangement was much the same as it had been four days after admission.

DISCUSSION

The patients included in this study were selected on the basis of ophthalmoplegia, but it is noteworthy that all of them exhibited some degree of nystagmus, ataxia and mental disturbance. This combination of clinical findings is so distinctive that there can be little doubt as to the accuracy of the diagnosis.

The ophthalmoplegia was surprisingly uniform. In all the patients, it consisted on admission of bilateral external rectus muscle weakness, which was usually asymmetrical in degree. Of the six patients who were sustained on glucose and saline before the institution of thiamine, the ocular paralysis became worse in all, progressing to an almost complete paralysis of gaze in two (Cases Nos. 3 and 6). In another of these patients (Case No. 4) a definite increase in the degree of ocular paralysis occurred within 24 hours after niacin alone was added to the purified diet, and in two (Cases Nos. 5 and 6) within 24 hours after the addition of multiple vitamins exclusive of thiamine. The possibility of an accentuation of a thiamine depletion by the addition of other vitamins deserves consideration in these three patients, especially in view of the experiments of Alexander, Pijoan, and Myerson (13), who claimed that the characteristic changes of Wernicke's encephalopathy could be produced in pigeons more easily if large supplies of other vitamins were added.

The most striking response to thiamine therapy in these patients was the rapid improvement in the ocular palsies, which cleared considerably within one and a quarter to six hours following parenteral administration of thiamine (after as little as 5 mgm. of this vitamin given to one patient). Nevertheless, it sometimes took several days for the residual weakness to disappear entirely. The miotic, nonreacting, pupils of one patient (Case No. 3), which developed while on glucose and saline only, returned to normal as the external ocular palsies cleared.

The rapid reversal of the ophthalmoplegia with thiamine makes it unlikely that these paralyses resulted from the structural lesion which is considered to be so characteristic of Wernicke's syndrome. Instead, it implies that this part of the clinical disorder was biochemical in nature. The pathological study of Riggs and Boles (4) lends support to this interpretation, for in five cases with oculomotor paralysis the ocular nuclei were not significantly damaged. Furthermore, Prickett (9) in a study of thiamine deficient rats and pigeons also noted a prompt reversal of the neurological disturbances after the administration of thiamine. Such dramatic response to thiamine, moreover, leaves little doubt as to the specificity of this vitamin for the ophthalmoplegia of Wernicke's syndrome. An important clinical lesson to be obtained from this material is that the ophthalmoplegia, which is an essential diagnostic criterion of Wernicke's syndrome, may be eradicated promptly by the administration of thiamine, either parenterally or possibly in the diet. Many of these cases are not then recognized as having Wernicke's syndrome.

Some degree of horizontal nystagmus occurred in all of the patients. Only in recent years has the significance of nystagmus been fully appreciated. de Wardener and Lennox (7), who studied 52 patients with Wernicke's syndrome, described nystagmus as the earliest ocular sign. In our patients during the glucose and saline control period, the horizontal nystagmus decreased only in association with an increase in ocular palsy and returned in full force after the ocular palsy was re-

[4] "Berocca C."

lieved by thiamine, indicating that it did not actually improve in the pre-thiamine period but had become less apparent because of increased paralysis of eye muscles. Improvement in nystagmus after thiamine, unlike ocular weakness, tended to be more gradual. Two patients (Cases Nos. 1 and 2), whose horizontal nystagmus did not improve over a four-day control period, showed definite decrease within three days after thiamine alone was added; in another (Case No. 8) there was marked improvement within one day of addition of thiamine alone. In one patient (Case No. 5) the marked vertical nystagmus present on admission showed no improvement after 24 hours on a group of vitamins exclusive of thiamine but cleared almost completely within 12 hours after thiamine only was added. In another (Case No. 7) the vertical nystagmus disappeared within five days after the institution of thiamine alone. These results suggest that the horizontal and vertical nystagmus also respond to thiamine. In four patients (Cases Nos. 1, 6, 7, and 9), however, nystagmus was still present after a month in the hospital. Two of these (Cases Nos. 6 and 7) failed to show any decrease in nystagmus after 27 and 18 days on food, respectively. Indeed, nystagmus has been noted to persist for months or years (17, 8) after an attack of Wernicke's syndrome.

Seven patients in this group had a markedly ataxic gait on admission, one (Case No. 4) showed only slight ataxia, and one (Case No. 6) could not be tested for character of gait because of the presence of a polyneuropathy which was so severe that he could not stand. The ataxia was essentially one of stance and gait and could seldom be well demonstrated in movements of the arms and legs individually, such as by finger-to-nose and heel-to-shin testing. Like the nystagmus, the ataxia was slow to improve, and of the seven patients who demonstrated it to a marked degree on admission, only one recovered from his ataxia completely, while six still showed it to a slight but definite degree when last examined. In no patient was there any improvement during the pre-thiamine control period, but in two (Cases Nos. 1 and 2), following a four-day control period, there was definite improvement within three days and one day, respectively, after thiamine alone was added. Similarly, Case No. 3, following a two-day control period, showed decrease in ataxia one day after thiamine was started and continued to improve on glucose and saline and thiamine only. In Cases Nos. 7 and 8, where glucose and saline and thiamine alone were given over a ten-day period, there was progressive improvement in the ataxic gait. It is noteworthy that in these five patients, the ataxia improved despite a low caloric intake. The ataxia, therefore, also seems to be related to a deficiency of thiamine, but because of the slow responses conclusions on this point are less certain than in the case of the ophthalmoplegia.

The mental component of the Wernicke syndrome presented the greatest difficulty in evaluation. Of the nine patients, only one (Case No. 4) was lucid on admission; however, he became increasingly confused during the period of glucose and saline administration and showed no improvement 48 hours after niacin and 24 hours after thiamine was started. Food was then given and in 12 hours he was again mentally clear; a day later, however, he developed an episode of confusion, which lasted 36 hours. It was impossible in this instance to determine whether improvement was due to a delayed effect of niacin or thiamine, to some other substance in food, to a spontaneous recovery from an unrelated confusional psychosis such as delirium tremens, or to some other factor.

The remaining eight patients all showed a profound degree of mental disorder on admission to the hospital. With only minor variations, certain features were common to all. When first seen the patients were alert, although not hyperactive or hallucinating. Often, their attitude was one of complete disinterest, rather than drowsiness. Spontaneous speech was minimal; they tended to answer questions in a perfunctory manner, with no desire to sustain a conversation. They were unable to focus their attention on any one topic, and would often suspend a conversation. The questions that were answered betrayed disorientation in time and place, misidentification of the people attending them, and complete inability to grasp the meaning of their illness or what was going on around them. In addition, many of their remarks were irrational, nor did these show any consistency from one moment to the next. If the examiner was persistent in his questioning, it was also obvious that retentive memory, especially for recent events, was already an important feature of the general mental disorganization.

The eventual outcome of the mental disturbance varied to some degree. One patient (Case No. 5) seemed to clear somewhat within 48 hours after receiving multiple vitamins and 12 hours after thiamine, and another (Case No. 8) became slightly less confused while maintained on glucose and saline and thiamine only. The improvement in these two patients was mainly in their orientation in place. In the other six patients, no change of this sort could be detected. If one measured the difference in mental state in terms of increased alertness, cheerfulness, ability to concentrate and sustain a conversation, and ready confabulation, then some degree of change occurred in most of the patients. Since this change occurred only after thiamine was added to the diet, it is possible that this aspect of the mental disorder was related to thiamine deficiency. With respect to memory defect and confabulation and to a lesser extent confusion, no significant improvement could be discerned in these patients within the periods of observation. Since these aspects of the mental disturbances characteristically resolve slowly, if at all (17), the periods of observation on the purified diet may have been too short to evaluate the effect of thiamine. This failure to improve after thiamine administration, therefore, does not exclude thiamine deficiency as a cause of the mental disturbances.

SUMMARY AND CONCLUSIONS

1. Nine patients with Wernicke's syndrome, characterized by ophthalmoplegia, nystagmus, ataxia, and mental disturbances were given a purified diet composed solely of glucose and minerals. Specific vitamins were added after appropriate intervals of observation.

2. Prior to the administration of thiamine, there was no improvement in any of the signs. More specifically, despite alcohol withdrawal, bed rest, and the addition of other vitamins (niacin, calcium pantothenate, pyridoxine, folic acid, ascorbic acid, riboflavin, B_{12}), the ophthalmoplegia progressed and the ataxia remained unchanged, while the nystagmus decreased only in association with an increase in ocular paralysis.

3. When thiamine only was added to the purified diet, the ophthalmoplegia cleared considerably in from one and a quarter to six hours; diminution in nystagmus and ataxia also occurred in some of the patients, but the change was more gradual; there was improvement in mental disturbance, but it was minimal in degree and consisted of increased attentiveness and capacity to maintain a conversation and greater ease of confabulation.

4. In view of these observations, there seems to be little doubt that the ophthalmoplegia of Wernicke's syndrome is related to a specific lack of thiamine. The nystagmus and ataxia also appear to be related to thiamine deficiency, but the evidence is less conclusive. No definite conclusions can be drawn regarding the relationship of the mental disturbances to the deprivation of thiamine or other vitamins.

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CARDIOVASCULAR AND RENAL ADJUSTMENTS TO A HYPOTENSIVE AGENT

(*l*-HYDRAZINOPHTHALAZINE: CIBA BA-5968: APRESOLINE)[1, 2]

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(Submitted for publication May 16, 1952; accepted July 30, 1952)

Since the work of Goldblatt, Lynch, Hanzal, and Summerville which related renal ischemia causally to hypertension (1), it has seemed logical to use renal vasodilators in the treatment of the syndrome. Yet the pyrogens are the only substances which heretofore have been demonstrated to increase consistently renal blood flow and these have not been found suitable for therapeutic use (2, 3). Chemical or hormonal compounds proposed as anti-hypertensive agents either exert a negligible effect on the renal circulation or reduce renal blood flow while lowering arterial pressure. These include the adrenergic blocking agent dibenamine (4, 5), the benzodioxanes (6), the ergot alkaloids (7), and the imidazolines (6, 8). Xanthine derivatives, of which aminophylline may be considered the prototype, induce renal hyperemia only in congestive cardiac failure (9, 10). The commonly used peripheral vasodilators—nicotinic acid, histamine, and the nitrites—similarly fail to augment renal blood (3, 11).

l-Hydrazinophthalazine (Ciba BA-5968 or Apresoline) is the basic chemical compound and one of several substituted compounds which have in common the properties of (*a*) increasing renal blood flow and (*b*) reducing arterial pressure in both animals and man (12–16).

It has been shown that this drug antagonizes the pressor action of epinephrine and to a lesser extent that of nor-epinephrine (14, 17–20). It abolishes the pressor effect brought about by centripetal vagus stimulation and thought due to serotonin (21), and it has an inhibitory effect against the pressor action of angiotonin and possibly other factors causally related to hypertension (22–24). It modifies the pressor response to the cold pressor test and the overshoot following the Valsalva maneuver (24). It causes increased coronary blood flow in the canine heart lung preparation (25). The site of action is unknown but it has been suggested that this drug reduces the outflow of sympathetic vasopressor impulses by central action (26).

It was the purpose of this study to measure and to evaluate certain cardiovascular and renal adjustments induced by this compound in normal and in hypertensive subjects.

METHOD

A total of 27 patients were studied. All were given *l*-hydrazinophthalazine in rapid single intravenous doses of 0.25 – 0.50 mgm./Kg./body weight. Basal determinations of blood pressure and pulse were made before administration of the drug and these were repeated every 30 seconds to five minutes for one to two hours thereafter, depending upon the rate of change.

Renal plasma flow (C_{PAH}) and glomerular filtration rate (C_{IN}) were simultaneously measured in 16 patients by the conventional clearance techniques (27).[4] All values before administration of the drug represent the average of at least three successive urine collection periods of 12–20 minutes each. Following administration of the drug several additional successive collections of similar duration were made.

Cardiac catheterization was performed in four healthy normotensive, non-hospitalized subjects and in two patients with hypertensive heart disease. Cardiac output, vascular resistance and work load were determined during the basal state and at intervals following administration of *l*-hydrazinophthalazine up to two hours. Cardiac output was measured by the usual determination of oxygen consumption and A–V oxygen difference.

[1] This project was supported (in part) by a grant from the National Heart Institute, U. S. Public Health Service, and by the Ciba Pharmaceutical Company.

[2] Presented (in part) at the 5th Annual Meeting of the Western Society for Clinical Research, January 26, 1952, Carmel, California.

[3] Fellow in Medicine.

[4] For the determination of inulin, auto-oxidation of glucose was substituted for destruction of glucose by fermentation, according to the modification of J. M. Little (J. Biol. Chem., 1949, **180**, 747).

Electrocardiograms were recorded serially in three normal subjects. In ten normal subjects skin temperatures were recorded simultaneously over six positions by means of an automatically recording potentiometer. Readings were made continuously in a constant temperature room at 22–33° Centigrade in the post-absorptive state for several hours before and after intravenous or intra-arterial injection of the compound.

RESULTS

1) *Renal function*

The renal plasma flow increased in 12 of 16 subjects, the average maximum increase being 38% (11–122%) (Figure 1). Of the four patients failing to demonstrate an increase in renal plasma flow, three had severe benign essential hypertension and manifested a pronounced and sustained fall in arterial pressure to shock levels in the recumbent position with consequent temporary but almost complete cessation of renal blood flow in two of the patients (Figure 2). The fourth patient had rheumatic heart disease with mitral stenosis and aortic insufficiency and was in incipient congestive cardiac failure. Of the 12 patients in whom the renal plasma flow increased, five were normotensive and the average maximum rise was 48% in this group. Four patients had essential hypertension with an average maximum

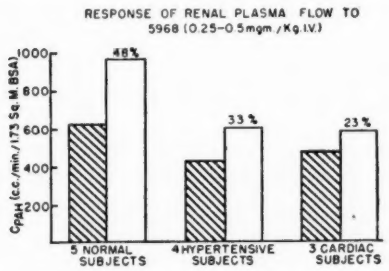


FIG. 1. RENAL PLASMA FLOW BEFORE (SHADED) AND AFTER ADMINISTRATION OF APRESOLINE

The hypertensive group does not include the three patients with clinical shock.

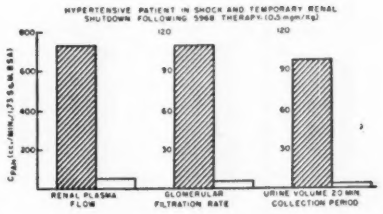


FIG. 2. APPARENT CESSATION OF RENAL BLOOD FLOW DURING PERIOD OF PRONOUNCED HYPOTENSION FOLLOWING THE ADMINISTRATION OF APRESOLINE IN C. B., A 28-YEAR-OLD MALE WITH ESSENTIAL HYPERTENSION

Two other patients responded similarly.

TABLE I

Renal plasma flow (C_{PAH}), *glomerular filtration* (C_{In}), *and filtration fraction* (*F.F.*) *in 16 subjects following the intravenous administration of Apresoline* (*Ciba-5968*)

Patient	Age	Diagnosis	Dosage 5968 I.V., mgm./Kg.	Before			After			
				C_{PAH}*	C_{In}*	F.F.	$C_{PAH\,(1)}$*	C_{In}*	F.F.	Max.* C_{PAH}
1. C. B.	21	Normal	0.25 (22.5 mgm.)	754	117	.16	936	105	.11	+ 24%
2. O. O.	39	Normal	0.5 (39 mgm.)	669	107	.16	1443	160	.11	+116%
3. J. W.	30	Normal	0.5 (40.5 mgm.)	814	156	.19	985	152	.15	+ 21%
4. W. C.	30	Normal	0.5 (35 mgm.)	566			780			+ 38%
5. J. B.	32	Normal	0.25 (21 mgm.)	541	92	.17	696	110	.16	+ 29%
6. M. B.	45	Ess. hypertension	0.5 (24 mgm.)	532	84	.16	705	86	.12	+ 33%
7. D. L.	31	Coarctation of aorta	0.5 (34 mgm.)	772	131	.17	860	110	.13	+ 11%
8. W. J.	63	Ess. hypertension	0.5 (22 mgm.)	246	63	.25	303	54	.18	+ 23%
9. M. S.	57	Ess. hypertension	0.5 (34 mgm.)	321	53	.16	458	50	.11	+ 43%
10. A. P.	59	Ess. hypertension	0.5 (31 mgm.)	252	42	.17	342	47	.14	+ 34%
11. C. B.	28	Ess. hypertension	0.5 (45 mgm.)	733	110	.15	51†	5	.10	− 93%
12. E. C.	62	Ess. hypertension Hyp. heart dis.	0.36 (22 mgm.)	320	83	.26	25†	3		− 92%
13. S. H.	46	Ess. hypertension	0.5 (29 mgm.)	380	67	.18	202†	24		− 47%
14. A. K.	23	RHD with M.S.	0.5 (24 mgm.)	538	97	.18	887	96	.11	+ 65%
15. L. T.	27	RHD with M.S. and A.I.	0.25 (17 mgm.)	536	106	.20	451	93	.21	− 16%
16. P. V. V.	59	Chr. pulm. dis. Cor. pulmonale	0.5 (33 mgm.)	356	116	.32	418	111	.27	+ 17%

* All clearances expressed as cc./min./1.73 Sq.M. BSA.
† Patient in shock.

TABLE II

Changes in arterial pressures in 20 subjects following the intravenous administration of Apresoline (Ciba-5968)

Patient	Age	Diagnosis	Dosage 5968 I.V. mgm./Kg.	Resting B.P.	Maximum B.P. change	Syst. diff.	Diast. diff.	Time of max. chg.	Time of normal return	Resting pulse rate	Max. pulse rate	Symptoms
1. C. B.	21	Normal	0.5 (42 mgm.)	110/60	130/50	+ 20	−10	8 min.	48 min.	72	104	Transient vertigo, frontal headache
2. G. D.	24	Normal	0.5 (49 mgm.)	110/80	85/35	− 25	−45	39 min.	68 min.	72	114	Flushing, tight throat, dizzy, yawning, nausea
3. E. F.	24	Normal	0.5 (36 mgm.)	110/80	105/55	− 5	−25	16 min.	120 min.	78	114	Left frontal headache, lethargy
4. F. R.	33	Normal	0.5 (39 mgm.)	130/85	120/60	− 10	−25	40 min.	60 min.	83	115	None
5. O. O.	39	Normal	0.5 (39 mgm.)	110/70	130/50	+ 20	−20	12 min.	72 min.	72	112	Slight chilling
6. J. W.	30	Normal	0.5 (40.5 mgm.)	136/88	168/86	+ 32	− 2	12 min.	25 min.	80	90	Vertigo, throbbing, headache, dyspnea, substernal oppression
7. W. C.	30	Normal	0.5 (35 mgm.)	120/60	116/46	− 4	−14	19 min.	50 min.			Transient light-headedness
8. J. B.	32	Normal	0.25 (21 mgm.)	118/66	None	None	None			60	84	Light frontal headache
9. D. L.	31	Coarctation of aorta	0.5 (34 mgm.)	130/80	125/60	− 5	−20	20 min.	57 min.	68	102	Palpitation, nausea, flushing, headache, dyspnea, precordial pain
10. M. B.	45	Essential hypertens.	0.5 (24 mgm.)	160/100	110/65	− 50	−35	29 min.	60 min.	84	120	Light-headedness, palpitation, flushing, weak, precordial pain, nausea, vomiting
11. W. J.	63	Essential hypertens.	0.5 (22 mgm.)	230/130	200/100	− 30	−30	6 min.	51 min.	80	104	Transitory dizziness
12. M. S.	57	Essential hypertens.	0.5 (34 mgm.)	160/110	130/80	− 30	−30	28 min.	55 min.	76	92	Drowsy, precordial pain, hyperventilation, nausea
13. A. P.	59	Essential hypertens.	0.5 (31 mgm.)	170/110	140/90	− 30	−20	10 min.	34 min.	76	108	Light-headedness, flushing, tinnitus, palpitation, diaphoresis
14. C. B.	28	Essential hypertens.	0.5 (45 mgm.)	190/140	100/68	− 90	−72	7 min.	120 min.	64	No Chg.	Headache, weakness, yawning, drowsiness
15. E. C.	62	Essential hypertens.	0.36 (22 mgm.)	210/140	85/45	−125	−95	16 min.	2 hr.			Angina, circumoral numbness, nausea, headache
		Hypertens. heart disease	0.25 (15 mgm.)	255/150	128/86	−128	−64	70 min.	2 hr.	80	110	
16. E. H.	46	Essential hypertens.	0.5 (29 mgm.)	190/110	84/40	−106	−70	14 min.	2 hr.			Nausea, headache, diaphoresis, circumoral numbness
17. M. C.	46	Essential hypertens. Hypertens. heart disease	0.37 (18.5 mgm.)	180/90	65/40	−115	−50	31 min.	2 hr.	74	44	Palpitation
18. A. K.	23	RHD with M.S.	0.5 (24 mgm.)	95/70	90/55	− 5	−15	60 min.	100 min.	80	148	Headache, substernal burning, palpitation
19. L. T.	27	RHD with M.S. and A.I.	0.25 (17 mgm.)	100/70	106/50	− 4	−20	20 min.	50 min.	60	68	Slight headache
20. P. V. V.	59	Cor pulmonale	0.5 (33 mgm.)	130/90	100/65	− 30	−25	37 min.	73 min.	96	108	Lethargy, flushing, tinnitus

rise of 33%. The lowest maximum rise was in patient No. 7 with coarctation of the aorta. The changes in renal plasma flow are statistically significant ($t = 4.08$, $p < 0.01$).

No consistent change in glomerular filtration rate occurred; it follows, therefore, that the filtration fraction was decreased (Table I). A diuretic action was a frequent but inconstant effect.

2) Arterial pressure

In eight normotensive subjects no consistent change in the level of systolic arterial pressure was noted while in the hypertensive group systolic pressures fell regularly following the administration of this compound (Table II). The fall in pressure in this group averaged 66 mm. Hg and was statistically highly significant ($t = 3.55$, $p < 0.01$).

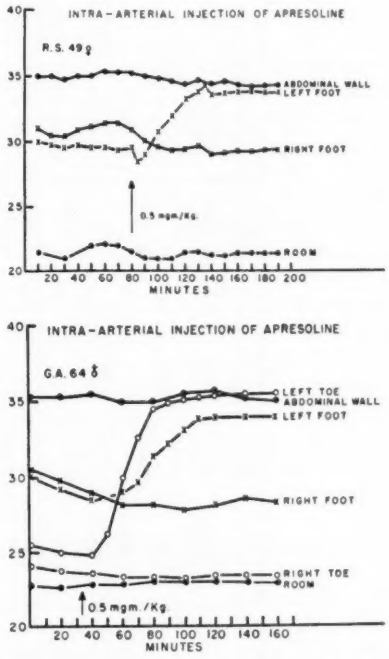
A diastolic blood pressure fall was a constant finding and in eight normotensive subjects diastolic arterial pressures decreased an average of 18 mm. Hg ($t = 3.18$, $p < 0.01$). The average maximum diastolic fall in the hypertensive group of patients was 50 mm. Hg (range 15–95). The maximum diastolic fall in both groups occurred within an average of 20 minutes and rarely returned to the pre-drug level within an hour, often not before two hours or longer. If the three patients are excluded who responded to the drug with sharp diminution of renal blood flow to shock levels, the average fall in diastolic pressure in the remaining hypertensive patients was 30 mm. Hg, excluding one patient in whom renal studies were not available but who demonstrated a severe reduction in arterial pressure from 180/90 to 80/46. Whenever measured, no fall in pressure was noted in the pulmonary circulation (see below).

Tachycardia was an almost constant occurrence although occasionally excessive bradycardia was observed. Frequent minor side effects were noted. Of these, palpitation, transient light headedness, frontal headache, flushing of the head and neck, nausea and vomiting, and circumoral numbness were most common. These symptoms were accentuated in the upright position and orthostatic accentuation of the hypotension was usually present.

With the exception of the sinus tachycardia previously noted, there were no electrocardiographic abnormalities observed throughout serial recordings in three normal subjects.

3) Skin temperature

Skin temperature studies were performed in eight patients, only one of whom was subjected to other Apresoline studies. The intravenous injection of the drug to five patients at constant temperature of 22–33°C. failed to increase the skin temperatures of the extremities. In one patient two separate intra-arterial injections of 0.10 mgm. (0.25 mgm./Kg.) were also ineffectual. In one patient with unilateral sympathectomy the temperature of the uninvolved extremity rose slowly to the level of the sympathectomized limb after a total of 20 mgm. (0.30 mgm./Kg.) had been injected into the femoral artery. In three other sub-



FIGS. 3a AND 3b. INTRA-ARTERIAL INJECTION OF APRESOLINE INTO THE LEFT FEMORAL ARTERY OF TWO SUBJECTS, WITH HIGH (a) AND LOW (b) RESTING TEMPERATURE OF THE LOWER EXTREMITY

All measurements were made through a seven-point automatic constant recording potentiometer with temperatures recorded every two minutes. Ordinate scale in °C.

TABLE III

Cardiovascular hemodynamics in four normal patients following the intravenous administration of Apresoline (Ciba 5968) (0.5 mgm./Kg., I.V.)

Pt.	Age		C.I. (*L./min./Sq.M. BSA*)	Chg. C.O. (%)	MPAP (*mm. Hg*)	TPPR (*dynes sec. cm.$^{-5}$*)	Chg. TPPR (%)	Arterial pressure (*mm. Hg*)	TSPR (*dynes sec. cm.$^{-5}$*)	Chg. TSPR (%)	LVW (*Kg.M./min./Sq.M. BSA*)	RVW (*Kg.M./min./Sq.M. BSA*)	LVW/RVW	Chg. LVW/RVW (%)
1. C. B.	21	Rest	3.22	+114%	8.5	109	−17%	110/60 (77)†	984	−56%	3.65	.393	9.29	−44%
		After 5968*	6.90		15	90		130/50 (77)	430		7.63	1.47	5.19	
2. G. D.	24	Rest	2.82	+138%	10	133	−50%	110/80 (90)	1200	−76%	3.65	.410	8.90	−55%
		After 5968*	6.71		12	67		85/35 (52)	291		5.00	1.16	4.31	
3. E. F.	24	Rest	2.52	+130%	10	179	−14%	110/80 (90)	1573	−62%	3.14	.862	8.68	−58%
		After 5968*	5.86		20	154		105/55 (72)	595		6.06	1.68	3.61	
4. F. R.	33	Rest	2.59	+ 65%	13	210	−25%	130/85 (100)	1611	−50%	3.72	.485	7.68	−35%
		After 5968*	4.26		16	157		120/60 (80)	811		4.90	.979	5.01	
Average				+112%			−27%			−61%				−48%

$$\text{Ventricular Work} = \frac{(\text{C.I.} \times 1.055)(13.6 \times \text{mean B.P.})}{1000}$$

$$\text{Total Vascular Resistance} = \frac{\text{mean B.P.} \times 1332}{\text{C.O. (c./sec.)}}$$

C.I.—Cardiac Index
C.O.—Cardiac Output
MPAP—Mean Pulmonary Artery Pressure
TPPR—Total Pulmonary Peripheral Resistance
TSPR—Total Systemic Peripheral Resistance
LVW—Left Ventricular Work
RVW—Right Ventricular Work

* At height of change.

† Figures in parentheses = mean arterial pressure.

jects 0.5 mgm./Kg. of Apresoline injected into the femoral artery caused a prompt rise in the surface temperature of the extremity involved (Figure 3).

4) Cardiac output

In four healthy normotensive subjects the cardiac output was sharply increased by *l*-hydrazinophthalazine (Table III, Figure 4). The maximum increase in cardiac output in the four cases occurred in 15 to 45 minutes after the drug was administered and then slowly declined. Cardiac output rose an average of 110%. The output increased disproportionately to the tachycardia so that the stroke volume increased an average of 66%. The mean pulmonary artery pressure changed slightly from an average of 11 mm. Hg to 16 mm. Hg. Because of the excessive rise in cardiac output the calculated total pulmonary resistance fell by an average of 27% when the appropriate formulae were employed. The total systemic resistance, on the other hand, decreased an average of 61% due to the fall in arterial pressure concomitant with a rise in output.

Because pulmonary artery pressures did not share in the peripheral vasodilatation the right ventricular work was disproportionately increased over the left ventricular work so that the ratio LVW/RVW was reduced by one-half.

In two patients with hypertensive heart disease, who had previously been in congestive failure, cardiac output increased only 17% (1.23 L./min.) and 21% (0.85 L./min.) respectively. In one patient pulmonary artery pressures rose from a mean of 21 to 51 mm. Hg while the arterial pressure fell from a mean of 190 to 100 mm. Hg. In the second patient pulmonary artery pressure remained essentially unchanged at 18 mm. Hg while the systemic pressure fell from a mean of 120 to 57 mm. Hg. The significance of these observations in subjects with a lowered cardiac reserve remains to be evaluated.

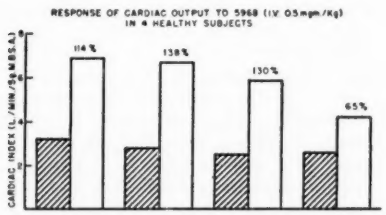


FIG. 4. A SHARP INCREASE IN BOTH STROKE VOLUME AND MINUTE OUTPUT OCCURRED IN FOUR NORMOTENSIVE SUBJECTS IMMEDIATELY UPON INJECTION OF APRESOLINE AND LASTED FOR APPROXIMATELY ONE HOUR

DISCUSSION

l-Hydrazinophthalazine lowers arterial diastolic pressure when administered intravenously and with equal dosage the fall in pressure is proportionately greater in hypertensive subjects than in normal individuals. Systolic pressure declined only in the hypertensive patients. Renal blood flow increased in all normal subjects but in contrast to the fall in pressure the increase was more striking in the normal than in patients with hypertension or heart disease. The difference between these groups was found to be statistically significant. These observations do not support the assumption that lowering of arterial pressure was mediated by an increase in renal blood flow. The two actions are apparently not interdependent.

The fall in arterial pressure was not caused by uniform vasodilatation. Pulmonary artery pressure did not fall and the intra*venous* injection of a hypotensive dose of Apresoline failed to cause a significant elevation in skin temperature when measurements were made over widely divergent areas. Its local vasodilating action could be demonstrated, however, by injecting large amounts of the drug intra-arterially. The fall in pressure is not the result of a lowered cardiac output in either normal or hypertensive subjects in whom adequate studies were available. It appears likely that selective vasodilatation, presumably of the splanchnic vessels, is responsible for the observed decrease in peripheral resistance.

The sharp increase in cardiac output and stroke volume in normal subjects together with the fall in pressure is of interest. The per cent increase in cardiac output exceeded the per cent increase in renal blood flow. Though measurements were not obtained simultaneously or in the same individuals, it does not appear likely that the results are random variations (Tables I, III). In the light of these observations a *relative decrease* in renal blood flow may be considered the renal pattern of this compound. That is, renal blood flow

increases, though to a lesser extent than the systemic blood flow.

The cardiovascular adjustments, characterized by an excessive decrease in total arteriolar resistance, are reminiscent of those observed following the administration of epinephrine in subjects in whom the vasoexcitor components of epinephrine have been blocked by dibenamine and who demonstrate Dale's blood pressure reversal in the face of increased cardiac output (28). It is to be stressed that a fall in arterial pressure in the face of increased cardiac output must represent marked peripheral vascular relaxation and for this reason Apresoline is considered a strong but selective vasodilator with properties resembling the vasodilator component of epinephrine, or epinephrine itself as contrasted with nor-epinephrine.

SUMMARY

1. *l*-Hydrazinophthalazine (Ciba BA-5968, Apresoline) was administered to 27 subjects in single intravenous or intra-arterial doses of 0.25 to 0.5 mgm./Kg. body weight.

2. A fall in systolic pressure occurred in hypertensive subjects only. Diastolic arterial pressures fell in all but two instances.

3. Renal blood flow (C_{PAH}) increased by an average of 40%, total peripheral blood flow (cardiac output) by 110% in the normal, resulting in a relative decrease of the fraction of the total blood flow made available to the kidneys.

4. No significant changes in skin temperatures were noted following intravenous injections. Intra-arterially administered, Apresoline caused a prompt and sustained rise in temperature of the involved extremity.

5. The compound represents a potent selective vasodilator with cardiac stimulating properties.

ACKNOWLEDGMENT

The authors wish to acknowledge the indispensable help of Mrs. Louise Green and Mr. Filimon Ukradaya.

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ADRENAL VIRILISM. II. METABOLIC STUDIES[1]

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(Submitted for publication June 25, 1952; accepted July 31, 1952)

INTRODUCTION

The clinical syndrome of virilism is well known and has been adequately described in the literature. In the female, when the syndrome is present at birth, the picture is that of pseudohermaphroditism, occasionally of such degree that genetic females are mistaken for males until gonadal biopsy reveals the presence of ovarian structures. When the syndrome develops later in childhood the features of virilism are superimposed upon normal female genitalia. In the male child, precocious puberty ensues. In all the children involved, there is ample evidence of excessive androgenic secretion; growth becomes accelerated; the skeleton matures early; there occurs enlargement of the clitoris in the female and of the penis in the male; and varying degrees of hirsutism and acne are encountered. The most common abnormality encountered in this syndrome is bilateral adrenal hyperplasia.

A rare variant of this condition occurs in newborn infants where in addition to the signs of virilism there is evidence of impaired ability to maintain normal serum concentrations of sodium and chloride. Such infants exhibit evidence of adrenal cortical insufficiency only in respect to salt and water metabolism. Many of the children in the past probably succumbed before the diagnosis was made. With recent advances in our knowledge of electrolyte metabolism, this clinical entity is being recognized more readily, and a gratifying response to therapy has been encountered.

The steroidal content of the urine in adrenal virilism has been the subject of intense investigation (1–9). The 17-ketosteroids and androgens are invariably elevated. The estrogen content of the urine is also increased (10, 11). Pregnanediol, pregnanetriol and other similar pregnane derivatives have also been isolated from the urine of patients with this condition (2, 7). The "corticoids" are usually normal but an occasional elevated value has been reported. It is this lack of increased production of the steroids, which are excreted as corticoids, which probably accounts in part for the difference in signs and symptoms of this condition as contrasted to the Cushing syndrome.

The adrenal cortices of patients with the adrenogenital syndrome appear to be secreting excessive amounts of androgen. Andrenocorticotropin has been administered to such patients in an attempt to ascertain how such adrenals respond to further stimulation (12–14). The purpose of this study was to elucidate, if possible, the underlying metabolic defect in adrenal hyperplasia and virilism.

METHODS AND MATERIALS

Seven patients with documented adrenogenital syndrome and proven bilateral adrenal hyperplasia were selected for these experiments. One male patient, aged two and a half years, presented the combined picture of virilism and "Addisonian-like symptoms"; the remaining six, ranging in age from 10 to 17 years, were female pseudohermaphrodites without abnormalities in sodium metabolism. To approximate a constant sodium chloride intake, the patients were placed on the Hospital salt-poor diet which has been designed to provide 0.5 Gm. of sodium daily and were given a measured 4 to 5 Gms. of added salt, so that the variations in the sodium chloride content of the diet would be reflected in but minor differences in the over-all sodium chloride intake. An indwelling catheter was inserted into the two and a half year old boy in order to insure complete urine collections. After a suitable control period, ACTH[4] in four divided daily dosages was administered to each patient for a three-day period.

The 17-ketosteroids were determined by the Holtorff-Koch (15) modification of the Zimmerman reaction; the formaldehydogenic steroids by the method of Daughaday,

[1] Aided by a Grant from the American Cancer Society as recommended by the Committee on Growth, National Research Council.

[2] Damon Runyon Senior Clinical Research Fellow.

[3] Squibb Fellow in Medicine.

[4] The ACTH was kindly supplied by Armour Laboratories and the cortisone and compound F by Merck, Inc.

Jaffe and Williams (16) as modified by Mason (17). For this procedure, an aliquot of the 24-hour urine specimen was acidified to pH 1 and allowed to stand at room temperature overnight before extraction with chloroform. Glucuronidase hydrolysis was not performed initially since the study was embarked upon before this method had been perfected. Subsequently, it seemed desirable to obtain strictly comparable data; hence this modification was not introduced. The analysis of urinary and serum sodium and potassium was performed by flame photometry. Completeness of the urine collections was checked by creatinine determinations.

After the three-day course of ACTH the patients were given cortisone or compound F[4] in an attempt to cause a reversal of the clinical state. These results have been presented in a separate publication (Jailer, Louchart, and Cahill [18]).

RESULTS

1. Virilism with Addisonian-like symptoms

Patient L. Y. was a two and a half year old male who in addition to virilism demonstrated "Addisonian-like symptoms." In order to maintain his serum sodium at normal levels it was necessary to administer 5 mg. per day of desoxycorticosterone acetate (DCA) intramuscularly and supplement his diet with 5 Gms. of added salt. Throughout the experimental period he was maintained on this regimen. The administration of a total of 17.5 mg. of ACTH for one day and 35 mg. of ACTH per day in divided doses for two days more resulted in a marked increase in urine volume and a diuresis of sodium. This was accompanied by a decline in his serum sodium from 133 to 124 mEq. within three days, loss of a pound in weight and a blood pressure fall from 120/90 to 90/60; consequently the experiment was terminated. Thus, evidence of adrenal cortical insufficiency supervened in spite of the fact that the urinary 17-ketosteroids and corticoids increased as a result of ACTH stimulation (Figure 1).

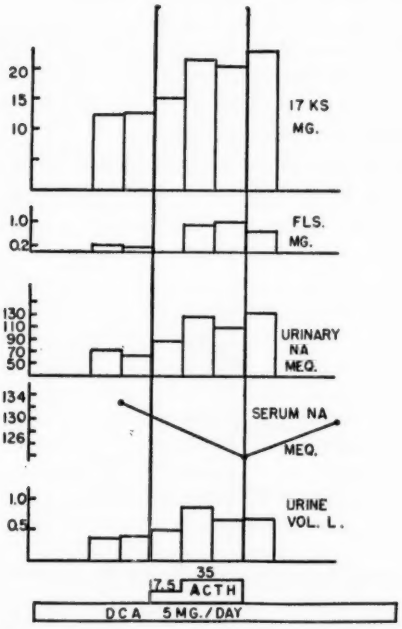

FIG. 1. THE EFFECT OF ACTH ON 17-KETOSTEROIDS (17-KS), FORMALDEHYDOGENIC STEROIDS (FLS), SODIUM AND URINE VOLUME IN PATIENT L. Y.

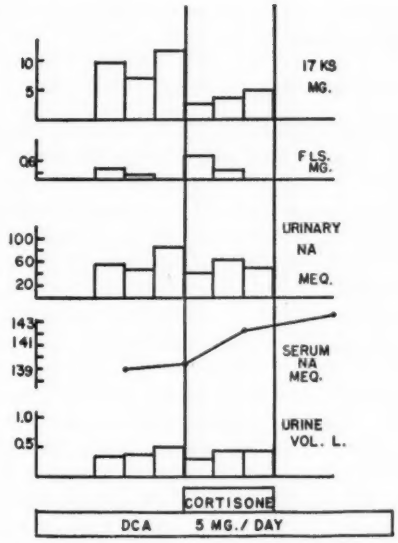

FIG. 2. THE EFFECT OF CORTISONE ON 17-KETOSTEROIDS (17 KS); FORMALDEHYDOGENIC STEROIDS (FLS), SODIUM AND URINE VOLUME IN PATIENT L. Y.

The subsequent administration of cortisone was without effect on the excretion of sodium but caused a marked decline in urinary 17-ketosteroids (Figure 2). While continuing cortisone it was possible gradually to withdraw the DCA and for more than a year this patient was maintained on as little as 12.5 cortisone per day intramuscularly with 5 Gms. added salt. Subsequent withdrawal of cortisone resulted in an exacerbation of all his

symptoms. At present he is continuing to receive daily cortisone.

2. Pure virilizing syndrome

Six pseudohermaphrodites, of which only four were on a constant salt intake, were given 75 to 100 mg. per day of Armour ACTH. In only one of these four patients (T. K.) was there minimal evidence of sodium retention as a result of the ACTH administration. In the other three, ACTH administration had no significant effect on sodium excretion (Figure 3). There was no suppression of urinary output during this period so that this lack of sodium retention could not be due to inhibition of the usual effects of ACTH by posterior pituitary contamination of the hormone preparation. Serum sodium levels were unaffected. There was no transient potassium diuresis. The administration of ACTH did not result in an increase in the excretion of the formaldehydogenic steroids (Figure 4) in six patients, and in only one patient (C. B.) was there a twofold increase in these urinary steroids. On the other hand, the administration of 100 mg. per day of the same batch of ACTH to a normal individual resulted in a fourfold increase in formaldehydogenic steroids. A clitorectomy under general anesthesia was subsequently performed on patient C.B. and on the first two days postoperatively there was no rise in the urinary corticoids.

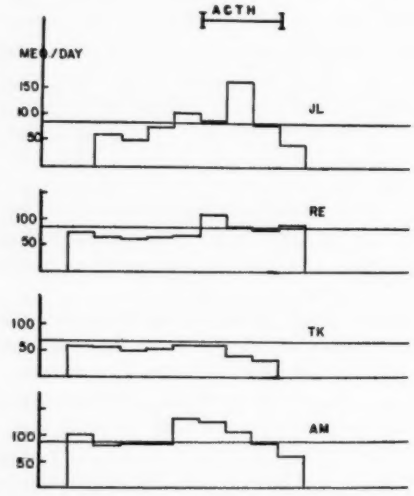

FIG. 3. THE EFFECT OF ACTH ON THE URINARY EXCRETION OF SODIUM

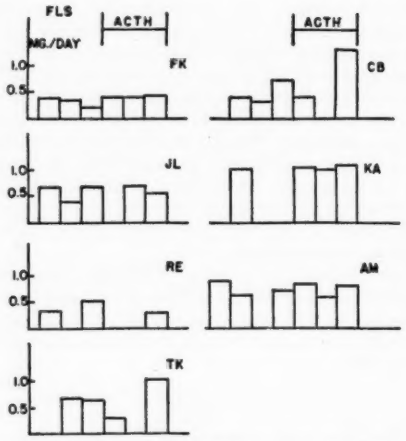

FIG. 4. THE EFFECT OF ACTH ON THE EXCRETION OF FORMALDEHYDOGENIC STEROIDS

Patient KA was a pseudohermaphrodite who was not included in the balance studies.

In only one of the four patients was there a marked decrease in circulating eosinophils during ACTH administration (Figure 5). This decrease in eosinophils has been amply shown to occur as a result of adrenal stimulation.

It was only in the sphere of urinary neutral 17-ketosteroids that there was evidence of a response

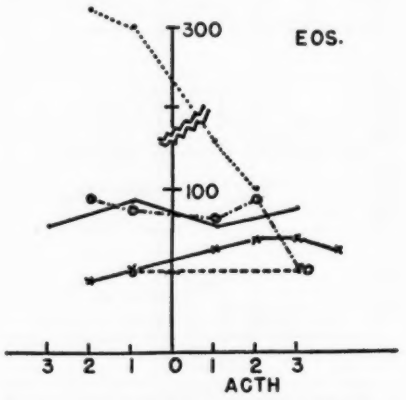

FIG. 5. THE EFFECT OF ACTH ON CIRCULATING EOSINOPHILS

The days to the left are control days.

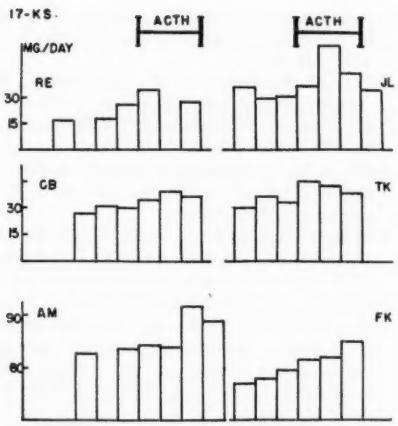

FIG. 6. THE EFFECT OF ACTH ON THE URINARY EXCRETION OF 17-KETOSTEROIDS (17 KS)

to pituitary stimulation. The elevated 17-ketosteroid values were further increased as a result of ACTH stimulation (Figure 6).

DISCUSSION

The administration of cortisone to patients with virilism due to adrenal hyperplasia results in a prompt decline in the urinary 17-ketosteroid excretion values. The mechanism for this effect is apparently due to the inhibition of pituitary ACTH secretion (11, 13, 18). Animal experiments have amply demonstrated that excessive cortisone administration can inhibit ACTH production (19).

The administration of ACTH to normal individuals results in adrenal stimulation as manifested by sodium retention, fall in circulating eosinophils, and increase in corticoids and 17-ketosteroids (20, 21). On the other hand, in patients with adrenal virilism, the injection of ACTH causes only the characteristic rise in urinary 17-ketosteroids but has minimal effects on corticoid excretion, eosinophils and salt retention. Bartter and his colleagues (13) have claimed that instead of the usual increased nitrogen excretion, patients with adrenal virilism may even retain nitrogen when given ACTH. Apparently, only the androgenic component of the adrenal can be stimulated in this disease.

In the one patient in whom the virilizing syndrome was associated with Addisonian-like symptoms the administration of ACTH was accompanied by increased sodium excretion as well as a rise in formaldehydogenic steroids. The fact that sodium diuresis ensued in spite of the continued administration of DCA suggests that a substance secreted by the adrenals may be considered a DCA antagonist in its overall effect, and is excreted in the "formaldehydogenic" fraction. The rise in formaldehydogenic steroids is in contrast with what obtains in the pure adrenal virilism, where these steroids are not increased by ACTH administration. It would appear that exogenous cortisone administration, by suppressing pituitary ACTH secretion, indirectly inhibits the production of this salt-losing steroid by the adrenal and therefore it is no longer necessary to administer DCA in order to maintain sodium balance.

There has been some speculation as to the underlying biochemical defect present in adrenocortical hyperplasia with virilism. The most obvious explanation would be that the adrenal cortex is secreting excessive amounts of androgens in addition to normal quantities of corticosteroids. However, if this were true it might be expected that further stimulation of such adrenals should result in increases in all moieties of adrenal steroids. However, this is not the case (13, 14). The administration of ACTH results in increases in only the androgenic components.

The administration of cortisone or compound F to patients with Addison's disease results in a rise in urinary 17-ketosteroid excretion (22) and therefore it may be assumed that these corticosteroids can be degraded to 17-ketosteroids. It may be postulated that in the hyperplastic adrenal of the adrenogenital syndrome the degradation of cortisone or compound F is deflected toward the 17-ketosteroid complex resulting in a temporary hypoadrenalism. The pituitary would in turn secrete excessive amounts of ACTH in an attempt to maintain circulating corticosteroids at a normal level. As a result of this, excessive amounts of androgens would be formed as a result of excessive degradation of the corticosteroids to the C-19 steroids. Therefore, any increment of ACTH would result in increases in androgenic secretion accompanied by no or minimal rises in corticosteroid liberation.

It is also tempting to speculate that the mechanism in Cushing's syndrome is somewhat different.

This syndrome is one of hyperadrenalism (23). In this condition, also, there is evidence that excessive quantities of ACTH are being secreted, but the degradation mechanism is different so that the corticosteroids are not converted to androgens.

SUMMARY

The administration of ACTH for three days to one patient with virilism and "Addisonian-like symptoms" resulted in an increased excretion of urinary sodium and a decline in serum sodium from 133 to 124 mEq. in spite of definite evidence of adrenal stimulation. The stimulation of the adrenals of patients with the pure virilizing syndrome was accompanied by increased 17-ketosteroid excretion; however, there was no significant sodium retention, fall in circulating eosinophils or rise in formaldehydogenic steroids.

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THE EFFECT OF EXPERIMENTAL VENOUS OBSTRUCTION ON SALT AND WATER DISTRIBUTION AND EXCRETION IN MAN

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(Submitted for publication May 19, 1952; accepted August 1, 1952)

An acute increase in local venous pressure produces a pooling of blood in the congested area and an alteration of fluid exchange at the capillary wall (1). This pooling occurs quickly and when produced experimentally by tourniquets applied to three extremities, has been shown to trap a volume equivalent to about 15 to 20 per cent of the total circulating volume within 10 minutes (2, 3). Elevation of venous pressure, according to Starling's fundamental hypothesis, will, in addition, initiate a filtration of fluid out of the capillaries into the local tissues (1, 2). This peripheral loss of fluid produces a decreased plasma volume and a rise in plasma protein concentration and hematocrit. The diminution in effective blood volume results in decreased venous return, a decreased cardiac output, a fall in blood pressure and a compensatory reflex vasoconstriction (4, 5). The increased plasma protein concentration combined with the fall in blood pressure will evoke a diffusion of interstitial fluid into those areas of the capillary bed not exposed to increased pressures. The ultimate stabilization of blood pressure will depend upon the extent to which these mechanisms compensate for the reduced circulating volume. The chain of events precipitated by the reduction in effective circulating volume is similar to that observed in dehydration and in hemorrhagic or traumatic shock (4–8).

The available evidence does not indicate whether or not, under the above circumstances, stores of electrolytes and water located outside of the fluid phase of the extracellular compartment contribute to the maintenance of plasma volume. Lands and Johnson suggested that hemorrhagic shock evoked a movement of intracellular water into the extracellular compartment (9). Stewart and Rourke concluded that blood loss was compensated, in part at least, by a movement of fluid from within the cells outward (10). In infants convalescing from dehydration due to diarrhea, a shift of water from the cells to the extracellular compartment has been noted (11). In conflict with these reports, Ashworth and Kregel found that cellular water increased at the expense of the extracellular volume after hemorrhage (12).

The use of recently described methods for measuring the distribution of water and electrolytes has suggested that significant stores of sodium and chloride are located outside of the fluid phase of the extracellular compartment (13–16). Such stores are labile and under appropriate stimuli may, in part, be transferred into this fluid phase (17–21). Using these techniques, an attempt has been made here to determine whether the systemic effects produced by local interference with venous flow include such an endogenous shift of electrolytes and water.

METHODS

The volume of the fluid phase of the extracellular compartment before and after the application of tourniquets was measured as the volume of distribution of inulin. Because the post-infusion method (13–16) for determining the inulin space permits only a single measurement in any 24-hour period, we have employed the difference method (20, 22, 23). The amount of inulin retained at any one instant is calculated as the difference between the total infused and the cumulative excretion. The latter technique permits serial space measurements over relatively brief periods of time so that acute changes may be detected. Both the post-infusion and difference methods afford identical volumes of distribution when urinary recovery of inulin is complete (20, 22–24).

Ten fasting adults were used in this study. Urine and plasma were collected for proper control determinations. An intravenous infusion containing 3.6 grams per cent of inulin and 100 mEq. per liter of sodium chloride was then administered from a calibrated burette accurate to 0.5 cc. In lieu of a priming injection, the infusion was started at

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a rate of 8 to 10 cc. per minute and then during the first 15 minutes gradually reduced stepwise to 0.8 cc. per minute.[3] This rate, maintained constant to within ± 1 per cent with a Bowman infusion pump, provided plasma levels of inulin between 30 and 50 mg. per cent. After four to four and a half hours of infusion, a period of time adequate to provide equilibrium distribution (13, 15, 16, 20), the bladder was catheterized and rinsed with distilled water and air. Directly before emptying the bladder, plasma was drawn for inulin and electrolyte analyses, and the volume of solution infused was read from the burette. A second control space was similarly measured five and a half to six hours after the start of the infusion.

After two control determinations of extracellular space were obtained, blood pressure cuffs were applied to both upper thighs in eight of 10 patients and inflated to pressures 5–10 mm. Hg below diastolic pressure so that venous flow was markedly obstructed. Systemic blood pressure was followed at frequent intervals and the pressure in the obstructing cuffs constantly adjusted to subdiastolic levels. The cuffs were kept in place for about three hours throughout which time no change was made in the rate of the inulin infusion. During this period additional measurements of extracellular space were made one to two hours and two to three hours after application of the tourniquets. The fluid level in the burette was read frequently to determine the constancy of the infusion rate. Plasma for inulin and electrolyte analyses was drawn from the non-involved arm before each bladder washout and at intervals of 30 minutes. In three patients several blood samples were drawn from the congested veins below the tourniquets simultaneously with the blood from the arm vein. After the final bladder washout the infusion was discontinued, the tourniquets removed and urine was collected for 18 to 24 hours to determine the completeness of recovery of the administered inulin. In two control patients serial space measurements were made at identical intervals without the application of tourniquets.

Each infusion and plasma and urine sample was analyzed for inulin four to 10 times by the resorcinol method of Schreiner (25). Spaces were calculated as the amount of inulin retained divided by the simultaneous plasma concentration in the non-congested upper extremity. Inulin clearances (GFR) were determined by the usual method (26), but the long intervals between bladder emptying provided only three to four periods (each of one to one and a half hours) in each patient.

Sodium and potassium concentrations were measured in an internally compensated Perkin-Elmer flame photometer. Chloride concentrations were determined by the Van Slyke and Hiller modification of the Sendroy method (27), plasma protein concentrations by the biuret technique (28), and venous hematocrit with the standard Wintrobe tube.

The electrolyte contained in the fluid phase of the extracellular compartment was calculated as the product of the inulin space and the plasma electrolyte concentration without corrections for Donnan's equilibrium. Throughout the experimental period, which lasted eight hours, each patient ingested two glasses of water, one during the control period and one about an hour after the application of tourniquets. During the tourniquet period a total of 12 to 14 mEq. of sodium chloride was infused, a variable proportion of which was retained.

[3] Technique, for attaining equilibrium distribution of inulin more quickly, suggested by Dr. Domingo Gomez.

RESULTS

Within minutes after the inflation of the tourniquets, marked rubor and congestion of the legs developed and persisted throughout the tourniquet-period. In three of the patients slight but definite pitting edema became apparent after one to two hours. Where pitting edema was not elicited, a thickening and induration of the subcutaneous tissue appeared. All subjects noted a sense of constriction and numbness but none complained of pain.

Of the eight patients so studied, three manifested moderate to marked reductions in blood pressure (Table I). Maximum falls in blood pressure occurred 60 to 90 minutes after inflation of the tourniquets and were accompanied by other signs of shock (salivation, nausea, vomiting in one patient, diaphoresis, pallor). In these patients the cuffs were temporarily partially deflated in order to maintain subdiastolic pressures. The shock-like reaction persisted for only three to 10 minutes despite the continued application of the tourniquets. During the last one and a half hours of the tourniquet period, the blood pressures gradually reverted toward control values. These three patients were the only subjects in the series whose control diastolic pressure exceeded 90 mm. of mercury. In five subjects little or no reduction of blood pressure occurred and no other untoward effects were noted.

The two control pre-tourniquet inulin spaces differed by less than 3 per cent. The control space listed in Table I is the second of these two pre-tourniquet determinations.[4] Following inflation of the tourniquets, each of the eight patients showed an increase in the volume of distribution of inulin. The increases recorded in Table I and Figure 1 averaged 16 per cent (1,400 cc.) with a range

[4] In one patient (Pan) only a single control space was measured after 4 hours of inulin infusion, but a similar control study performed subsequently demonstrated that equilibrium had been attained within 4 hours.

TABLE I

The effect of experimental venous obstruction on blood pressure, plasma protein concentration, inulin space, plasma electrolyte concentration, glomerular filtration rate and electrolyte excretion

Subject	Time* (min.)	Blood pressure (mm. Hg)	Total protein (gm. per cent)	Inulin space (cc.)	Serum electrolyte concentrations Na (mEq./l.)	K (mEq./l.)	Cl (mEq./l.)	Total electrolyte in ECF Na (mEq.)	Cl (mEq.)	Inulin clearance (cc./min.)	Urine flow (cc./min.)	Electrolyte excretion Na (μEq./min.)	K (μEq./min.)	Cl (μEq./min.)	Comment
Wee	Control	165/95	7.8	8,570	143	4.7	109	1,220	934	64	.48	11	24	19	Transient shock at 60–65 min.
	22	148/96	7.5		140	4.5	107								
	82	115/70	8.3		148	4.7	111								
	119	125/80	7.8	9,320	146	4.9	112	1,360	1,040	43	.38	4	27	9	
	188	144/90	7.5	10,200	137	4.8	106	1,400	1,080	58		2	20	10	
	Total increase			1,630				180	144						
Par	Control	110/70	6.9	7,500	136	4.3	112	1,020	840	61	.49	361	650	517	No untoward effects
	60	106/70	7.5		133	4.4	112								
	115	110/80	8.0		132	4.2	108								
	147		7.4	8,400	136	4.3	109	1,140	916	59	.52	445	420	525	
	Total increase			900				120	76						
Col	Control	110/80	6.7	7,420	132	4.4	113	980	839	85	2.78	321	42	361	No untoward effects
	62	110/84	7.3	7,740	135	4.3	111	1,040	859	65	.76	124	18	140	
	123	104/76	7.4	8,700	139	4.5	110	1,210	957	50	1.27	36	16	51	
	Total increase			1,280				230	118						
Bro	Control	190/110	7.3	9,210	146	3.4	109	1,350	1,000	49	2.3	366	17	232	Transient shock at 90–95 min.
	20	190/130													
	60	164/126	7.5		145	3.3	106								
	96	60/45	7.5		142	3.8	108								
	110	100/80													
	134	116/80	7.1	10,700	147	3.3	108	1,570	1,160	32	.60	58	11	60	
	Total increase			1,490				220	160						
Pan	Control	100/60	6.8	6,600	137	4.4	114	904	753	75	.96	117	32	82	No untoward effects
	64				149	4.4	122								
	127		8.3		147	4.4	113								
	189		8.0	8,030	143	4.3	113	1,150	907	65	1.28	113	22	88	
	Total increase			1,430				246	154						
Oss	Control	140/90	6.8	9,470	135		105	1,280	994	85	1.01	216	66	264	Transient shock at 85–90 min.
	45	138/80	7.5		138		104								Copious vomiting at 105–150 min.
	65	110/78	7.2	10,300	151	5.0	104	1,560	1,070	60	.67	120	58	160	
	85	88/68													
	105	110/74	7.5		140		107								
	120	130/80													
	141	150/90	7.5	9,950	133	5.0	104	1,320	1,040	71	.45	15	56	70	
	† *Total increase*			480				40	46						
Ban	Control	130/76	7.4	12,200	140	4.1	107	1,710	1,310	83	.91				No untoward effects
	56	122/70	7.1		138	3.6	110								
	118	114/76	7.0		138	3.4	111								
	156	116/76	7.2	14,800	142	3.7	108	2,100	1,600	82	.47				
	Total increase			2,600				390	290						
Ben	Control	110/76	8.6	12,000	136	3.0	105	1,630	1,260	106	.56	33	2.2	28	No untoward effects
	44	106/70	8.1		135	3.1	105								
	81	100/78	8.6	12,800	132	2.9	106	1,690	1,360	101	.51	48	1.7	39	
	119	110/80	8.4		130	2.9	104								
	153	110/80	8.3	13,100	133	2.8	103	1,740	1,350	111	.36	11	4.8	21	
	Total increase			1,100				110	90						
	Control patients—no tourniquets applied														
Hall	Control	95/60	6.7	8,850	144	4.3	113	1,270	995	103	1.3	277	54	292	
	63		6.3	8,640	143	4.1	110	1,240	950	100	3.0	233	81	272	
	115	unchanged	6.3	8,750	144	4.3	111	1,260	972	101	3.2	224	66	241	
	Total change			−50				−10	−23						
Bro	Control	190/110	6.7	11,300	138	3.7	108	1,560	1,220	56	2.1	248	25	250	
	62	unchanged	6.7	11,300	138	3.8	109	1,560	1,230	56	1.8	249	16	278	
	131		7.3	11,200	136	3.9	109	1,520	1,220	51	1.5	222	15	238	
	Total change			−100				−40	0						

* Time represents that number of minutes after the application of tourniquets.

† In this patient 65 minutes of venous congestion induced an expansion of the inulin space of 830 cc. and an increment of total extracellular sodium and chloride of 280 mEq. and 76 mEq., respectively. The subsequent fall accompanied a period of repeated and copious vomiting.

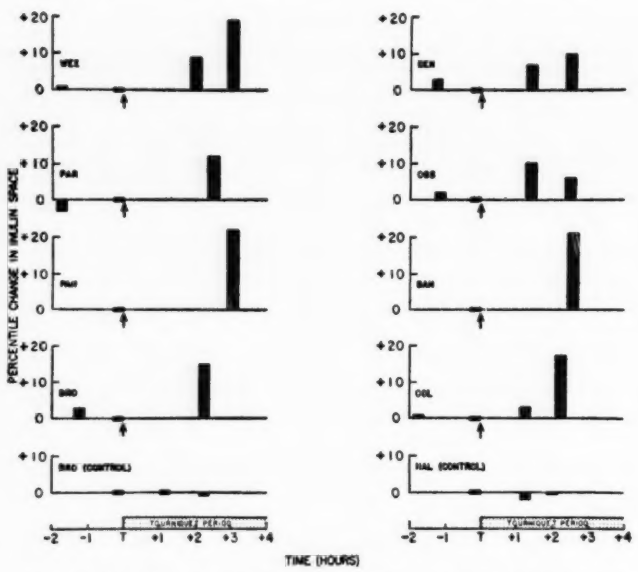

FIG. 1. CHANGES IN INULIN SPACE BEFORE AND AFTER APPLICATION OF TOURNIQUETS IN EIGHT EXPERIMENTAL SUBJECTS AND DURING COMPARABLE TIME INTERVALS IN TWO CONTROL SUBJECTS.

Arrows represent time of tourniquet application.

from 9 to 21 per cent. The maximum increase in the inulin space occurred two to three hours after tourniquet application. In one patient (Oss) the maximum increase occurred after one hour of venous obstruction; this patient, however, vomited profusely during the latter part of the tourniquet period.

Between 30 and 60 minutes after inflation of the tourniquets, the plasma inulin concentration increased in five of the eight patients to levels of 5 to 15 mg. per cent above control values. Thereafter the inulin concentration remained fairly constant or decreased slightly. Three patients showed no significant change in the plasma inulin concentration. In the three subjects in whom simultaneous plasma inulin concentrations were measured above and below the tourniquets, the latter concentration rose more slowly but finally approached

TABLE II

Detailed data including the plasma inulin concentrations, the quantity of inulin infused, excreted and retained, and the calculated inulin spaces from two typical experiments

Subject	Time from onset of infusion (*hr.-min.*)	Inulin plasma concentration (*mg. per cent*) a	Inulin infused during period (*gm.*) b	Inulin excreted during period (*gm.*) c	Inulin retained during period (*gm.*) b − c	Inulin retained − cumulative (*gm.*) Σ(b − c)	Inulin space (*cc.*) Σ(b − c)/a
Col	4–6	38.1	10.23	7.37	2.86	2.86	7,510
	5–6	38.7	2.05	2.04	.01	2.87	7,420
	5–17			Blood pressure cuffs applied			
	6–19	44.0	2.53	1.99	.54	3.41	7,740
	7–20	45.6	1.93	1.37	.56	3.97	8,700
Hal	(Control subject—cuffs not applied)						
	5–21	32.0	13.55	10.73	2.82	2.82	8,800
	6–34	33.8	2.15	2.05	.10	2.92	8,640
	7–26	33.8	1.79	1.75	.04	2.96	8,750

within 1 to 5 mg. per cent of the systemic plasma concentrations. The amount of inulin retained during the tourniquet period was proportionately greater than the coincident increase in systemic plasma concentration and, therefore, each patient evinced an expansion of the inulin space (Table I). In two patients in whom tourniquets were not applied no change in inulin space was detected during comparable time periods (Figure 1 and Table I—control patients). Table II records the detailed inulin data from two typical experiments.

Post-infusion urinary collections were complete in eight of the 10 patients studied. The inulin recoveries in these eight cases averaged 99.6 per cent with a range from 96.8 to 104 per cent.

Serial measurements of plasma protein concentration during the tourniquet period revealed increases in six of the eight patients (Table I). These changes were maximal after one to two hours of reduced venous flow and thereafter in four of these six subjects the protein concentration tended to return toward normal (Table I, Figure 2).

In five of the eight patients, no significant changes in plasma sodium or chloride concentrations were evident during the tourniquet period.

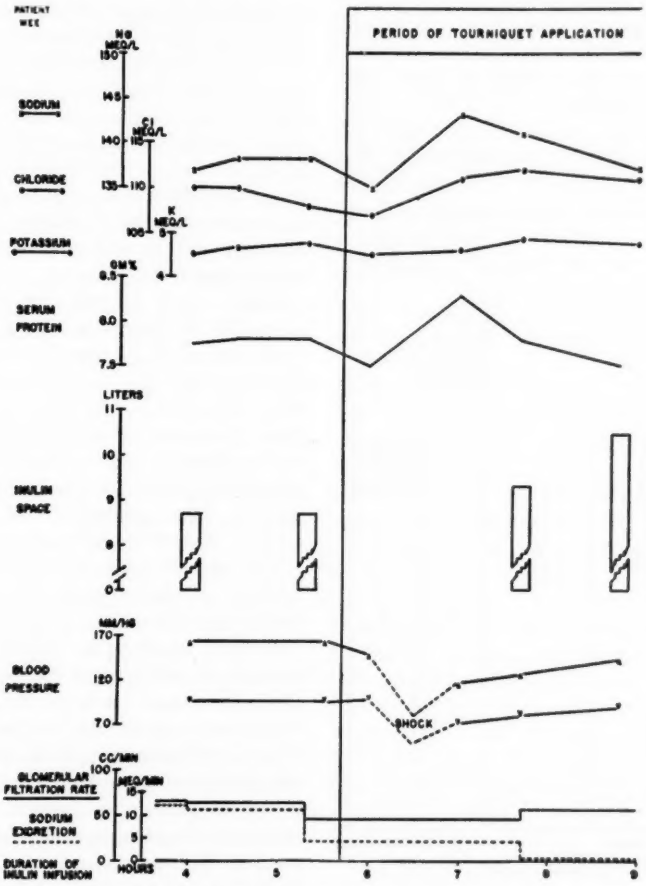

FIG. 2. SIMULTANEOUS CHANGES IN PLASMA ELECTROLYTE CONCENTRATIONS, PLASMA PROTEIN, INULIN SPACE, BLOOD PRESSURE, GFR AND SALT EXCRETION IN ONE TYPICAL EXPERIMENTAL SUBJECT

In three patients (Wee, Pan, Oss), however, the plasma sodium concentrations increased one hour after the application of the tourniquets and then gradually returned to control or subcontrol levels (Table I, Figure 2). Similar, but smaller changes in chloride concentrations were noted in two of these patients. The increase in sodium and chloride contained in the inulin space averaged 190 and 140 mEq., respectively (Table I, Figure 3).

A decrease in glomerular filtration rate (GFR), of more than 15 per cent, was noted during the tourniquet period in five of the eight patients (Table I). This decrement was most marked in the first hour of venous obstruction and was associated with a reduction in blood pressure in three patients. In two of these five subjects the GFR approached control values during the latter phase of the tourniquet period.

Five of seven patients evinced a marked decrease in sodium excretion following the application of tourniquets (Table I). Generally, this reduction was associated with a simultaneous decrease in GFR. However, in three patients in whom the filtration rate remained unchanged or increased during the latter phase of the tourniquet period, the rate of sodium excretion continued to decrease (Table I, Figure 2). The changes in chloride excretion were generally similar to those noted in sodium excretion. No consistent alterations were detected in the rate of potassium excretion. The rate of urine flow remained essentially unchanged in four subjects (Wee, Par, Pan, Ben), fell transiently in one (Col), and showed a sustained fall in three (Bro, Oss, Ban) (Table I).

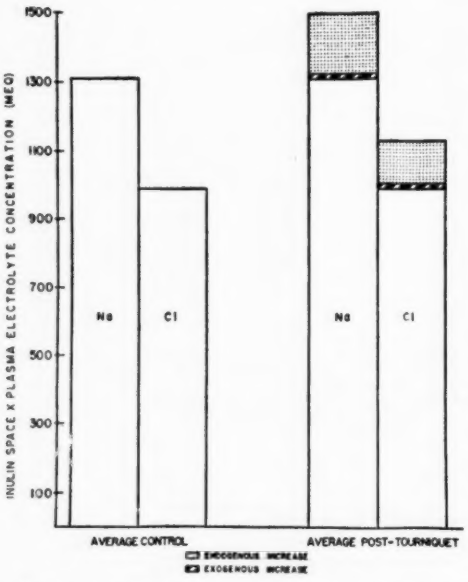

FIG. 3. AVERAGE INCREASE IN ELECTROLYTE CONTAINED IN THE INULIN SPACE AFTER TWO TO THREE HOURS OF REDUCED VENOUS FLOW

DISCUSSION

In evaluating the inulin space expansions noted during the tourniquet period, four possible sources of methodological error must be considered: *1*) a failure to attain the maximum volume of distribution of inulin (equilibrium distribution) prior to the application of the tourniquets; *2*) errors in the analyses of the urine, plasma or infusion samples; *3*) an increase in renal dead space during venous congestion; *4*) a state of disequilibrium produced by the experimental conditions and characterized by a concentration of inulin in the interstitial fluid higher than that of plasma.

Figure 1 and the examples shown in Table II reveal that the maximum volume of distribution of inulin was achieved after four to four and a half hours of infusion. Two control space determinations before the application of tourniquets and three consecutive space measurements in the control patients in whom venous flow was not obstructed agreed within 3 per cent. These findings indicate that uniform distribution had been achieved before the inflation of the tourniquets.

The multiple analyses of the urine, plasma and infusion provided a mean concentration with a standard deviation of $\pm$ 2 per cent. In the first prolonged equilibrating period in which large amounts of inulin were infused and excreted, a 2 per cent error in both quantities may produce a significant error in the calculated control space. Since subsequent changes in space depend upon the amount of inulin retained during the specific period in question, the absolute change in the inulin space will not be significantly influenced by any error in the calculated volume of the control space. In the interval periods about 2 gm. of inulin were infused and excreted; 2 per cent errors in determining these quantities could produce a maximal

expansion of about 200 cc. To account for the average expansion of 1,400 cc., an error of 20 to 25 per cent in overestimating the amount infused or in underestimating the amount excreted in any specific post-tourniquet period would be required. Such consistent discrepancies hardly appear plausible.

In the three subjects in whom venous congestion induced a sustained fall in urine flow, it is conceivable that a significant increase in the amount of inulin contained within the renal dead space occurred, thereby artificially augmenting the calculated space. However, two control studies have revealed that comparable falls in urine flow induced by pitressin may artificially expand the inulin space by only 100 to 300 cc. The dead space error may, therefore, account for only a small fraction of the space increase in these three subjects.

In the five subjects who showed a fall in glomerular filtration rate, increases in plasma inulin concentration occurred about 30 to 60 minutes after inflation of the tourniquets. That this rise in plasma concentration was not immediately distributed throughout the inulin space is suggested by the slower rise in inulin concentration in the venous blood drawn from the congested extremities. This discrepancy between the plasma concentration in the non-congested and congested areas probably resulted from the fact that considerable quantities of plasma ultrafiltrate were accumulated in the congested legs before the rise in inulin concentration occurred. However, since all space calculations were based on the higher plasma concentration in the non-congested area, any delay in the even distribution of this increment in concentration could only obscure an expansion in the inulin space. The stabilization of the inulin concentration during the last one and a half hours of the tourniquet-period in these five subjects and the absence of any significant change in inulin concentration in three subjects suggests that virtual equilibrium distribution was again achieved before the final space measurements.

The conceivable sources of error do not explain the consistent expansion in the volume of distribution of inulin after two to three hours of venous congestion in the lower extremities. That this increase was not a result of inulin metabolism is confirmed by the completeness of urinary recovery.

The arguments which imply that the volume of distribution of inulin affords a reliable estimate of the extracellular fluid volume have been previously presented (13–16, 20, 22, 23, 29). The concept of a homogeneous, entirely fluid, extracellular compartment with precise, sharply defined boundaries undoubtedly represents an oversimplification. It has been contended that certain extracellular areas are rendered inaccessible to the inulin molecule by a difference in the architecture of the underlying medium (30, 31). Relatively solid phases as contained in bone and connective tissue (tendon) are probably not included in such space measurements (32). The recent demonstration of the identity of the volume of distribution of inulin with that of sucrose (23), mannitol (33), ferrocyanide (22), and radiosulfate (34) suggests, however, that a finite and measurable fluid phase of the extracellular compartment probably exists. It is in this sense—that of a fluid phase in rapid exchange with the plasma and with the chemical composition of an ultrafiltrate of plasma (13)—that the terms extracellular fluid and interstitial fluid are used in this paper.

During the two to three hour period of reduced venous return, the total amount of salt administered in the inulin infusion equaled 12 to 14 mEq. Although the majority of these patients showed a reduction in salt excretion and thereby retained much of this amount, the positive exogenous balance can account for at most 5 to 10 per cent of the total increase in electrolyte contained in the inulin space. Accordingly, averages of about 180 mEq. of sodium, 130 mEq. of chloride and 1,300 cc. of water appear to have been transferred into the fluid phase of the extracellular compartment from endogenous sources during this period (Figure 3). The precise source of this salt and water is not clear. Significant quantities of salt (predominantly chloride) and water may be associated with the solid phase of connective tissue (35, 36). Large stores of sodium are located in the crystal structure of bone; salt and water are contained in the gastrointestinal tract. The salt and water may derive, in part at least, from any one of these sources. In addition, an unknown fraction may be furnished by cells.

It is unlikely that venous obstruction so alters tissues in the congested area as to induce a local outpouring of salt and water. Most observers have concluded that muscle anoxia and injury in-

duce a loss of intracellular potassium and a reciprocal gain of sodium (37, 38). The absence of an increase in plasma potassium or of a potassium diuresis suggests that no such cellular change occurred. Furthermore, the amount of salt available in the congested extremities (muscle cells are singularly free of sodium chloride [35]) appears inadequate to augment the extracellular fluid electrolyte by as much as 16 per cent. It is suggested, therefore, that the body tissue above the tourniquets may have supplied the bulk of endogenous salt and water. The amount of sodium chloride demonstrated to be contained outside of the inulin space is entirely adequate to supply 180 mEq. of sodium and 130 mEq. of chloride. Recent studies indicate that this degree of depletion would exhaust about one-fourth of the sodium and one-third of the chloride located outside of the inulin space (14, 15, 20, 39).

The increases in plasma protein concentration and hematocrit observed in the early stage of the tourniquet period are in accord with previous observations that tourniquets applied on the extremities at subdiastolic pressure produce a rapid reduction in plasma volume (1, 3). Whether any decrease in circulating volume, regardless of etiology, will initiate a movement of salt and water into the fluid phase of the extracellular compartment remains to be determined. Previous observations have suggested that DCA, cortisone, ACTH, and whole adrenal cortical extract produce similar shifts (17–19, 21, 40). The possibility, therefore, exists that this acute redistribution is similarly mediated through adrenal activity.

The tendency for the blood pressure to rise toward normal and for the serum protein concentration to fall in several subjects during the latter phase of the tourniquet period implies that some reconstitution of plasma volume was being accomplished. This reconstitution was abetted by a decreasing rate of plasma loss as local tissue pressure increased in the congested extremities (41). In addition, the endogenous expansion of the extracellular fluid volume may have enhanced the compensatory transfer of interstitial fluid into the depleted plasma in those areas of the capillary bed not exposed to increased pressure. Other investigators have demonstrated that the volume of the extracellular compartment in part determines the resistance of experimental animals to acute reductions in blood volume. Prior dehydration exaggerates the lethal effect of bleeding in dogs whereas prior saline infusions protect the animals (6, 42, 43).

Reductions of GFR have been noted following interference with local venous return (44, 45). These changes have been attributed to the fall in blood pressure and to the generalized vasoconstriction in which the renal parenchyma is involved (4, 5, 43). Because only two prolonged periods were obtained after tourniquet application, it is possible that in several subjects acute falls in GFR were obscured. The reduction in sodium excretion correlated only partly with the GFR as exemplified in those subjects where late in the tourniquet period sodium excretion continued to fall despite unchanged or increasing filtration rates (Table I, Figure 2). This phase of enhanced tubular reabsorption of salt coincided with the period of maximum redistribution of salt. Thus, these mechanisms, redistribution and retention, may be interdependent and complementary in retaining or augmenting extracellular fluid salt.

CONCLUSIONS AND SUMMARY

1. The volume of distribution of inulin, plasma electrolyte concentrations, plasma protein concentration, glomerular filtration rate, rate of electrolyte excretion, and blood pressure were measured serially before and during a two to three hour period in which tourniquets were applied at subdiastolic pressures to the thighs of eight human subjects. Comparable measurements were made at similar time intervals in two control subjects on whom tourniquets were not applied.

2. In each of the eight experimental subjects, two to three hours of venous congestion induced an expansion of the inulin space. The increase averaged 16 per cent with a range from 9 to 21 per cent. No consistent changes in plasma electrolyte concentrations were detected.

3. In five of the eight subjects, a reduction in glomerular filtration rate occurred. This reduction was most marked in the early phase of venous congestion. In five of seven subjects, a reduction in the rate of sodium excretion occurred. This reduction correlated only in part with the fall in glomerular filtration rate.

4. Transient falls in blood pressure were apparent in three of the eight subjects. Six sub-

jects showed a slight to moderate increase in plasma protein concentration which reached maximal values after one to one and a half hours of venous congestion. Subsequently, in four subjects, these concentrations tended to return toward normal.

5. Two control subjects showed no change in GFR, inulin space, blood pressure, plasma electrolyte concentrations, or electrolyte excretion.

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RELATIONSHIP OF THE RENAL CLEARANCES OF T-1824 AND OF ALBUMIN IN SOME PATIENTS WITH PROTEINURIA

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(Submitted for publication January 31, 1950; accepted August 1, 1952)

Studies of the mechanism of proteinuria and particularly of albuminuria require the application of accurate methods to the determination of the concentrations of individual proteins in plasma and urine. Electrophoresis is not practical because of the very large number of analyses necessary. The availability of human plasma albumin (i.e., fraction V) has permitted extensive use of immunological techniques (1–3). However, the small but significant amounts of globulins present in this fraction and the tedious procedure required in the preparation of antiserum warranted the investigation of possible alternative methods for the estimation of the renal clearance of albumin.

The blue dye T-1824, used clinically for the estimation of plasma volume, is bound in plasma principally to the albumin fraction (4); it does not appear in urine in significant amounts unless there is a significant proteinuria. On the basis of these two facts it has been suggested that the clearance of the dye might be a measure of the clearance of albumin. The first investigation of the clearance of the dye was carried out by Luetscher some years ago (5); the clearance of the dye was proportional to the clearance of the total protein and approximately four times as great. Since the albumin clearance was nearly four times the total protein clearance it is evident that there was good correlation between the dye and albumin clearances. Data were also given directly comparing the dye and albumin clearances in two instances: in the first the dye clearance was 0.37 ml./min., in the second the dye clearance was 0.75 ml./min. compared to the albumin clearance of 0.66 ml./min. The albumin concentrations were determined electrophoretically. More recently, Allen and Orahovats (6) have investigated the clearance of T-1824 in normal dogs. From a comparison of their data for T-1824 in dogs with the data on protein excretion in normal human subjects obtained by Gunton and Burton (7) they suggested that the renal clearances of albumin and of T-1824 might be identical.

The present report describes the results of direct comparisons of the renal clearances of the dye and of albumin in patients with the nephrotic syndrome. Results are also given of direct comparisons in some of these patients after changes in the plasma albumin concentration had been obtained by the infusion of concentrated human plasma albumin.[4]

METHODS

a. Concentration of T-1824 in plasma. A previously described acetone extraction method was used to determine the concentration of T-1824 in plasma (8, 9). The increment of optical density (ΔD) for a given increment of plasma dye concentration (ΔC) is a function of protein concentration but follows the Bouguer-Beer Law for any given protein concentration. In some of the experiments reported below, the plasma protein concentration changed significantly from one period to the next following the administration of human plasma albumin or, in one experiment, the administration of blood. In these experiments it was necessary to determine $\Delta D/\Delta C$ for each sample of plasma. This was done as follows: From each plasma sample containing dye two 1 ml. aliquots were taken. To one, A, was added a known amount of dye in 0.2 ml. of 0.85 per cent NaCl. To the other, B, was added 0.2 ml. of 0.85 per cent NaCl. From this point on, the acetone extraction procedure was followed. The optical densities of the acetone extracts of A (D_A) and of

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TABLE I

Comparison of clearances of albumin and T-1824 in J. R., male, aged 5 years

Date	Time of urine collection	Urine flow	Plasma concentration		Renal plasma clearance		
			Albumin	T-1824	Albumin	T-1824	$\frac{C_{T\text{-}1824}}{C_{alb}}$
		ml./min.	*gm./100 ml.*	*μgm./ml.*	*ml./min.*	*ml./min.*	
Dec. 2, 1948	10:26 a.m.–12:09 p.m.	0.25	0.38	16.8	0.68	0.43	0.63
	–12:43	0.47	0.38	14.2	0.99	0.70	0.71
	– 1:00	0.59	0.38	13.4	1.25	0.94	0.69
(Control)	– 2:10	0.40	0.385	12.2	0.81	0.56	0.70
	– 3:30	0.60	0.39	10.2	0.81	0.61	0.75
	– 4:06	0.78	0.39	8.65	0.93	0.80	0.86
	– 5:43	0.58	0.39	7.55	1.00	0.70	0.70
	– 7:08 p.m.	0.51	0.39	6.60	0.81	0.63	0.77
Dec. 14, 1948	9:00 a.m.– 9:45 a.m.	25 gm. albumin intravenously					
	10:02 a.m.–10:31 a.m.	2.48	1.72	15.6	2.37	1.99	0.84
	–11:48	2.29	1.67	13.5	1.90	1.83	0.96
(after 25 gm. albumin)	–12:25 p.m.	2.00	1.45	11.8	2.19	1.93	0.88
	– 1:17	2.54	1.37	11.0	2.11	1.85	0.88
	– 1:56	2.80	1.31	10.2	1.96	1.76	0.90
	– 2:55	1.80	1.24	9.30	2.07	1.91	0.92
	– 3:20	2.56	1.18	8.60	2.18	2.02	0.93
	– 3:40	1.60	1.17	8.35	2.28	1.94	0.85
	– 4:58	0.90	1.12	7.65	1.85	1.79	0.97
	– 5:53	0.62	1.02	6.65	2.05	1.82	0.89
	– 6:53	0.48	0.92	5.75	1.63	1.42	0.87
	– 8:17 p.m.	0.33	0.81	4.85	1.49	1.31	0.88

$B(D_B)$ were read in the Coleman Model 6A clinical spectrophotometer in 12×75 mm. cuvettes at $\lambda = 620$ mμ with the instrument set at zero with distilled water. $\Delta D = D_A - D_B$. This value of ΔD was then plotted against the amount in μgm of dye added to 1 ml. of plasma A. A straight line was drawn through the point and the origin, and the concentration of dye equivalent to D_B was read from this line. In a large series of determinations on the control plasma of nephrotic patients the difference between distilled water and an acetone extract of plasma free of dye was found to range from 0.000 to 0.003 optical density units. Therefore no significant error was introduced by the assumption that the optical density of an acetone extract of plasma was due solely to dye. This is of importance because residual dye from previous injections was present in the plasma of several patients at the beginning of the experimental period.[5]

[5] When plasma volumes were estimated during the course of an experiment the procedure previously described (7) was used for the determination of the relative dye concentrations. To obtain the absolute dye concentrations required for the calculation of clearances the reference solution used was standardized by comparison with a solution containing a known concentration of dye. Between 1 and 5 ml. of 0.5 per cent T-1824 was injected per liter of plasma volume; the resultant maximum dye to albumin molar concentration ratio was approximately 1:8. Urine collections for the clearance studies were started no sooner than 20 minutes after the injection of the dye.

In general, duplicate analyses of the plasma concentrations of dye could not be made because of the limited amount of blood that could be safely removed from each patient. However, about 10 per cent of the determinations were repeated because of apparent discrepancies with other analyses; these duplicates showed no more than 2 per cent variation.

b. Concentration of T-1824 in urine. Urine samples, if cloudy, were cleared by centrifugation; extraction with acetone was not necessary. To accurately measured aliquots of urine containing dye, there were added 1.0 ml. of molar phosphate buffer of pH 7.0 and enough 0.85 per cent NaCl to make the final volume 5.0 ml. The volume of urine was chosen so that the final optical densities were above 0.10 in 12×75 mm. cuvettes at $\lambda = 620$ mμ with the instrument set at zero with water. Standard curves were prepared by adding a known amount of dye to a buffered control urine; thus $\Delta D/\Delta C$ was found for the urine for each experiment. Blanks were troublesome because it was not possible to determine accurately what fraction of the blank was contributed by the naturally occurring urine pigments and what fraction was contributed by dye still being excreted as the result of previous injections of dye. Ideally, the blank contributed by the urine pigments should have been calculated on the basis of excretion per minute; this was not possible and no blank correction was made. Because of the relatively high dye clearances in these patients, the effect of this omission on the calculated dye clearances is to make them too high by at most 5 per cent. Where lower clearances

TABLE II

Comparison of clearances of albumin and T-1824 in A. McE., female, aged 5½ years

Date	Time of urine collection	Urine flow	Plasma concentration		Renal plasma clearance		
			Albumin	T-1824	Albumin	T-1824	$\frac{C_{T\text{-}1824}}{C_{alb}}$
		ml./min.	*gm./100 ml.*	*μgm./ml.*	*ml./min.*	*ml./min.*	
Dec. 3, 1948	9:40 a.m.–10:45 a.m.	37.5 gm. albumin intravenously					
	1:37 p.m.– 2:19 p.m.	1.31	2.79	17.6	1.94	1.37	0.71
(after 37.5 gm. albumin)	– 3:04	1.02	2.69	16.4	1.55	1.21	0.78
	– 3:37	0.75	2.63	15.5	0.64	0.50	0.79
	– 3:50	1.15	2.62	15.2	1.00	0.78	0.78
	– 4:34 p.m.	1.05	2.60	14.7	1.18	1.02	0.86
Dec. 4, 1948	9:41 a.m.–11:08 a.m.	0.67	1.80	25.2	0.67	0.50	0.75
	–12:12 p.m.	0.73	1.67	23.0	0.75	0.54	0.72
	–12:48	0.50	1.60	21.9	0.82	0.58	0.70
	– 1:29	0.98	1.55	20.9	0.81	0.57	0.70
	– 2:01	1.72	1.55	20.3	1.08	0.76	0.71
	– 3:24	0.95	1.54	19.5	0.72	0.54	0.75
	– 4:03	1.51	1.53	18.7	0.80	0.58	0.73
	– 4:45	0.95	1.53	18.2	0.80	0.57	0.71
	– 6:01	0.40	1.53	17.3	0.76	0.51	0.67
	– 7:03	0.53	1.52	16.3	0.82	0.57	0.70
	– 8:38	0.38	1.52	15.3	0.54	0.42	0.77
	–10:10	0.37	1.51	14.3	0.54	0.40	0.75
	–11:47 p.m.	0.36	1.50	13.5	0.52	0.38	0.74
Dec. 5, 1948	– 2:55 a.m.	0.27	1.50	12.4	0.41	0.32	0.78
	– 5:59	0.27	1.48	11.2	0.40	0.33	0.82
	– 9:56 a.m.	0.28	1.47	9.95	0.42	0.39	0.92

of dye than those reported here are found, a pigment blank correction becomes essential.

Another possible source of error is the change in the absorption coefficient for the dye with changes in the protein to dye molar concentration ratios. Fortunately, under the conditions of the experiments reported here the albumin to dye molar concentration ratios were between 1 and 100, in a range where there is very little change in the dye absorption coefficient (10). Since the Coleman Model 6A clinical spectrophotometer isolates a band approximately 35 mμ wide, changes in absorption coefficient at a given wavelength setting and shifts of absorption maxima are much less evident with this instrument than with the Beckman quartz spectrophotometer.

Replicate determinations of dye concentrations in urine, on some occasions two or three days apart, showed in the absence of marked bacterial growth no greater variation than ± 1.5 per cent.

c. Albumin concentrations in plasma and urine. Albumin concentrations were estimated by an immunological procedure similar to that described by Kunkel and Ward (11). Since T-1824 is bound to albumin in plasma and presumably in urine, the possibility that T-1824 might interfere with the antigen-antibody reaction was examined. T-1824 was added to an albumin solution in 0.85 per cent NaCl in a molar concentration ratio of dye to albumin of 14 to 1; antiserum was added to this solution and to a solution of albumin of the same concentration but without T-1824. The presence of T-1824 made no detectable difference in the amount of precipitate formed; no dye was found in the antigen-antibody precipitate. The possibility of interference by NH_4^+ was eliminated by the fact that addition of NH_4Cl to albumin prior to reaction with the antiserum did not result in any greater optical density after reaction with ninhydrin than when NH_4Cl was not added.

d. Patients. See (3, 12).

e. Urine collections. All specimens of urine were spontaneously voided.

RESULTS

In Table I are given comparisons of the dye and albumin clearances in patient J. R. on a control day and after the intravenous administration of albumin on another day. Table II presents similar data obtained from an experiment on patient A. McE.: in this experiment clearances were estimated over a period of approximately 48 hours after the administration of 37.5 gm. of human serum albumin. Figure 1 summarizes the data on comparisons between dye and albumin clearances. It will be noted that the two clearances are not in an exact 1 to 1 ratio and that nearly all the T-1824 clearances are smaller than the corresponding albumin clearances. The means, standard devia-

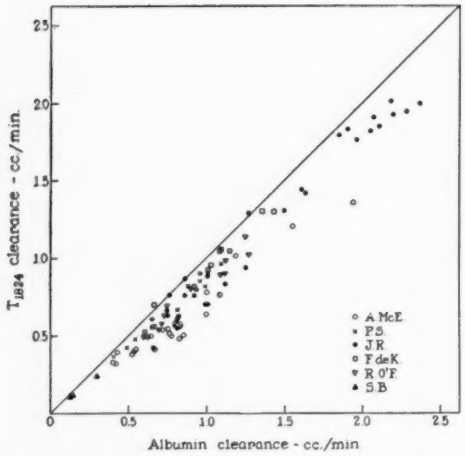

FIG. 1. RELATIONSHIP OF THE T-1824 AND ALBUMIN CLEARANCES

The straight line is the locus of a ratio of 1.0.

tions, and coefficients of variation of the clearance ratios are given in Table III for each experiment on each patient and for all the data taken together. The extent of variability found is not surprising since the ratios include the errors of four analyses: urine and plasma T-1824 concentrations and urine and plasma albumin concentrations. For a given experiment in a given patient the standard deviation of the clearance ratios is less than the standard deviation of all the clearance ratios taken together.

DISCUSSION

It is evident from the data that there is a reasonably good agreement between the clearance of the dye T-1824 and the clearance of albumin when the albumin concentrations are estimated by the immunological procedure used here. This result was expected because T-1824 is preferentially bound to albumin in plasma and presumably also in urine. The fact that the clearances are not identical warrants brief comment.

The most obvious explanation of the discrepancy in the clearances is that there is a systematic error in the albumin clearances: the albumin clearances may be too high. This could occur either if the values for the plasma concentration of albumin were too low or if the values for the urine concentration of albumin were too high; the alternatives are not mutually exclusive. The probability that this explanation is correct is lessened by the following facts. The antigen used to prepare the anti-serum contains approximately 2 per cent alpha-globulins. As pointed out elsewhere (3) the renal clearance of alpha-globulins is much smaller than the clearance of albumin. If the immunological method determines some alpha-globulin as albumin the clearance values for albumin would be too low rather than too high.

TABLE III

Summary of the comparisons of the renal plasma clearances of T-1824 and albumin

Patient	Date	Number of periods	Range of albumin clearances	T-1824 clearance / Albumin clearance		
				Mean	Standard deviation	Coefficient of variation
			ml./min.			*per cent*
A. McE. (after albumin)	Dec. 3–5, 1948	21	0.40–1.94	0.75	±0.057	± 7.6
	Dec. 21–22, 1948	5	0.67–0.99	0.64	±0.066	±10.3
J. R. (control)	Dec. 1–2, 1948	8	0.68–1.25	0.73	±0.066	± 9.0
(after albumin)	Dec. 14–15, 1948	12	1.49–2.37	0.90	±0.041	± 4.6
(before and after blood)	Feb. 2–3, 1949	12	0.65–1.61	0.91	±0.081	± 8.9
P. S. (after albumin)	Dec. 14–15, 1948	10	0.40–1.09	0.88	±0.039	± 4.4
R. O'F. (after albumin)	Dec. 1–2, 1948	13	0.60–1.27	0.84	±0.055	± 6.5
F. deK. (after albumin)	Dec. 13–14, 1948	10	0.61–1.42	0.94	±0.059	± 6.9
S. B.	Dec. 8, 1948	3	0.13–0.30	0.85	—	—
All cases		94	0.13–2.37	0.83	±0.101	±12.2

It is possible that the discrepancies are related to the existence of two or more plasma albumins. Luetscher found that albumin was homogeneous on electrophoresis at pH > 7; at pH 4.0 there were two distinct components (13). Cohn, Hughes and Weare working with crystallized serum albumin reported that they had confirmed Luetscher's findings (14). Hughes has identified a distinct fraction, mercaptalbumin, in crystalline albumin (15). If native albumin or albumin which has been carried through the ethanol fractionation procedure is a mixture of two (or more) proteins there is no assurance that these proteins have identical clearances. Nor is there any assurance that T-1824 is bound in exactly the same manner to the two proteins.

With respect to T-1824, it is unlikely (though not impossible) that a large fraction of the dye is bound to other proteins such as the alpha-globulins at the *in vivo* dye to protein concentration ratios of our experiments. Rawson (4) has demonstrated that T-1824 added to whole plasma migrates with albumin during electrophoresis at pH 7.4 when the molar ratio of T-1824 to albumin is less than 14 to 1. Another possibility is that some of the T-1824 may be reabsorbed by the tubular cells independently of the "reabsorption" of albumin. Finally, some of the dye may be destroyed (e.g., by reduction of the azo groups) on passage down the tubules.

Regardless of which, if any, of the above possibilities is correct, the data indicate the extent to which the clearance of T-1824 may be depended on as a measure of the clearance of albumin.

SUMMARY

The renal plasma clearances of the blue dye T-1824 and of human plasma albumin have been estimated simultaneously in patients with the nephrotic syndrome. In 94 periods in 6 patients the ratio, T-1824 clearance/albumin clearance, averaged 0.83 (standard deviation, ± 0.101). Concentrations of albumin in plasma and urine were estimated by means of an immunological procedure. The T-1824 clearance should be useful as a substitute for the albumin clearance where direct methods for the albumin clearance are not available or where it is desirable to estimate a large number of clearances. The T-1824 clearance is proportional to, though not identical with, the albumin clearance over a wide range of albumin clearance values. Possible explanations of the discrepancy between the clearances are discussed.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the technical assistance of Miss Dora M. Newell.

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MECHANISMS CONTRIBUTING TO UNRESPONSIVENESS TO MERCURIAL DIURETICS IN CONGESTIVE FAILURE [1, 2]

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(Submitted for publication July 11, 1952; accepted August 1, 1952)

The ultimate failure of edematous cardiac patients to respond to mercurial diuretics is well established. Although the older literature ascribed this to renal tubular accommodation to mercury, some patients have received mercurials for many years without manifesting such acquired tubular resistance. More recently, because of the potentiation of mercurial diuresis in previously unresponsive patients by pre-treatment with ammonium chloride, resistance to mercurials has been attributed to chloride depletion (1, 2). However, acidotic potentiation of mercurial diuresis may persist after the elevated serum chloride level has returned to normal (3). Moreover, mercurial-resistant patients may not exhibit hypochloremia and some hypochloremic patients continue to have good mercurial diureses (2).

Since organic mercurials act by decreasing renal tubular reabsorption of filtered electrolyte and water, any factor which increases such reabsorption will tend to antagonize the diuretic effect of mercurials. The present communication is an attempt to analyze mercurial resistance in cardiac patients in the light of the severely impaired renal excretion of salt and water, resulting from the changes in cardiovascular-renal hemodynamics, hormonal balance, and other mechanisms affecting renal tubular activity in congestive failure.

One of these factors, decreased glomerular filtration rate, reduces the quantity of filtered sodium and chloride presented to the tubules. Recent studies have shown that whenever filtration is decreased without change in tubular function the resulting glomerulo-tubular imbalance is associated with a more complete reabsorption of sodium (4, 5, 6, 7). Thus, late in congestive failure, the progressive impairment of glomerular filtration may finally so diminish the quantity of electrolyte filtered that virtually all of the sodium reaching the tubules is reabsorbed; consequently, negligible diuresis results even after depression of tubular activity by the usual dose of a mercurial.

This relationship between the electrolyte load delivered to the tubules and the response to mercurials was evaluated in a series of mercurial-resistant subjects by studying the influence on mercurial diuresis of measures which increase the quantity of sodium filtered. The results suggest that a reduced filtered load of electrolyte, as well as other factors, may contribute to the state of mercurial resistance.

METHODS AND MATERIALS

The subjects were nine edematous and one edema-free cardiac patients, maintained on a low salt diet, who were no longer responsive to mercurials despite adequate digitalization, bed rest, and pre-treatment with ammonium chloride. Seven were patients with rheumatic heart disease, uncomplicated by known renal disease. Two were patients with arteriosclerotic heart disease. One (J. B.) was an edema-free, digitalized, young hypertensive, in whom a marked drop in filtration rate and renal plasma flow had been produced during a six-week period on the rice diet with repeated administration of mercurial diuretics until no further response was elicited (8).

With the resting patients in the post-absorptive state, renal clearances of mannitol and sodium para-aminohippurate, and urinary outputs and serum concentrations of sodium and chloride were determined, before and after achieving a maximal response to a 2 ml. intravenous injection of either Mercuzanthin or Thiomerin. Immediately thereafter, an effort was made to increase the quantity of filtered sodium, either by the intravenous administration of 0.72 gm. of theophylline ethylene diamine (aminophyllin), or the rapid infusion of a concentrated sodium chloride solution or a sodium chloride-sodium lactate mixture. All the determinations then were repeated. The clearance techniques and chemical methods

[1] Supported in part by grants from the National Heart Institute, U. S. Public Health Service; Martha Hall Foundation, Montefiore Hospital; Campbell Pharmaceutical Co.; Eli Lilly & Company; Wyeth, Inc.; and G. D. Searle & Co.

[2] Presented in part at II Inter-American Cardiological Congress, Chicago, 1948.

[3] Rosenstock Memorial Fellow, 1947–1949.

TABLE I

Typical diuretic response to Mercuzanthin in patient K. B. with moderate impairment of renal hemodynamics

Period	R.P.F.	G.F.R.	Serum Na	Serum Cl	Na filt.	Na excr.	Cl filt.	Cl excr.	Urine vol.
	ml./min.	*ml./min.*	*mEq./l.*	*mEq./l.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*ml./min.*
Control	221	81.6	133	99.2	10.9	0.002	9.03	0.003	1.09
Mercurial	229	89.6	132	98.0	11.2	0.730	9.21	0.762	6.91

employed have already been described (8). In two patients, the augmentation of mercurial diuresis by aminophyllin or by concentrated sodium infusions was studied in separate experiments at three and six week intervals, respectively. In one patient, aminophyllin was administered also after previous potentiation of mercurial diuresis by sodium chloride. Control experiments and other variations in experimental design are discussed below.

RESULTS

In the tables, unless otherwise indicated, renal hemodynamic and electrolyte and water excretion data for "control" periods represent averages of at least two 20 to 30 minute clearance periods before administration of the mercurial, whereas "mercurial" periods represent those of maximal diuretic response or, in the case of completely unresponsive patients, clearance periods 60 to 120 minutes after injection of the mercurial. The renal hemodynamic data are not corrected for body surface area.

In Table I, the typical changes in renal plasma flow, glomerular filtration rate,[4] quantity of sodium and chloride filtered,[5] and electrolyte and water excretion following intravenous injection of 2 ml.

[4] Mannitol was used as an index of filtration rate when these experiments were performed. It has since been established that some reabsorption of this hexose does occur (9).

[5] Calculated from serum electrolyte levels, corrected for Donnan equilibrium, in this and all succeeding tables.

TABLE II

Effect of the increased G.F.R. following aminophyllin injection on mercurial diuresis in mercurial resistant patients

Patient	Period	R.P.F.	G.F.R.	Serum Na	Serum Cl	Na filt.	Na excr.	Cl filt.	Cl excr.	Urine vol.
		ml./min.	*ml./min.*	*mEq./l.*	*mEq./l.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*ml./min.*
	Control	115	41.1	122	84.3	5.01	0.002	3.82	0.004	2.78
C. R.	Merc.	99	36.1	120	84.6	4.32	0.005	3.37	0.021	1.86
	Aminoph.	139	46.5	119	84.6	5.51	0.165	4.34	0.285	4.69
	Control	197	55.8	114	92.4	6.36	0.002	5.70	0.006	1.94
E. S.	Merc.	164	46.4	112	88.7	5.20	0.026	4.59	0.050	2.23
	Aminoph.	228	59.6	114	85.1	6.78	0.389	5.64	0.614	8.04
	Control	175	60.9	116	82.4	7.06	0.002	5.05	0.001	2.23
B. G.	Merc.	148	56.0	120	81.5	6.75	0.003	4.43	0.002	1.94
	Aminoph.	212	66.3	120	78.8	7.96	0.020	5.23	0.062	3.47
	Control	211	60.7	135	88.8	8.29	0.149	6.07	0.150	8.98
J. B.	Merc.	188	53.2	137	90.1	7.49	0.199	5.38	0.216	5.44
	Aminoph.	269	75.8	137	89.9	10.5	0.896	7.65	0.807	14.1
	Control	80	45.7	117	89.5	5.38	0.001	4.57	0.003	0.80
A. B.	Merc.*	81	44.1	115	85.7	5.05	0.014	4.20	0.045	2.14
	Aminoph.	115	61.0	115	85.4	7.00	0.227	5.76	0.371	7.11

* Thiomerin 2 ml. The other patients received Mercuzanthin 2 ml.

TABLE III

Comparison of effect of aminophyllin and Mercuzanthin plus aminophyllin in chronic congestive failure

Patient	Period	R.P.F.	G.F.R.	Serum Na	Serum Cl	Na filt.	Na excr.	Cl filt.	Cl excr.	Urine vol.
		ml./min.	*ml./min.*	*mEq./l.*	*mEq./l.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*ml./min.*
E. G.	Control	—	65.0	139	—	8.99	0.004	—	—	1.60
	Aminoph.	—	90.0	142	—	12.8	0.020	—	—	4.37
H. G.	Control	247	71.2	132	91.8	9.49	0.009	7.34	0.031	1.44
	Merc.	231	69.5	132	91.5	9.27	0.125	7.13	0.203	2.19
	Aminoph.	347	90.4	132	89.1	12.0	0.738	9.00	0.939	7.45

of Mercuzanthin in a mercurial-responsive patient (K. B.) are presented. This patient, whose glomerular filtration rate was only moderately decreased, excreted negligible quantities of sodium and chloride during the control periods but following the injection of the mercurial exhibited a marked sodium, chloride, and water diuresis. In contrast, patient C. R. (Table II), whose glomerular filtration rate and filtered electrolyte loads were markedly reduced, responded negligibly to this diuretic. However, when 0.72 gm. of aminophyllin were injected intravenously 90 minutes after the mercurial had been given, there was significant augmentation of filtration rate, renal plasma flow, and urinary excretion of sodium, chloride, and water.

In this laboratory, similar injections of aminophyllin alone generally have resulted in relatively small increases in the electrolyte excretion of patients in severe chronic congestive failure with functionally decreased renal hemodynamics. In Table III, such a response to aminophyllin alone in patient E. G. is compared with the response to aminophyllin following a mercurial in patient H. G., who had equivalent serum electrolyte levels and filtration rate. The latter exhibited only a moderate diuretic response to Mercuzanthin until 0.72 gm. of aminophyllin were injected. Then, with the marked increase in glomerular filtration rate and renal plasma flow, the increase in sodium and chloride excretion was much greater than that usually observed after aminophyllin alone. Similar enhancement of mercurial response was observed in previously resistant patients, J. B., E. S., and A. B., in whom both filtration rates and renal plasma flows rose following injections of aminophyllin (Table II).

In several patients (C. R., E. S., and A. B.), despite the improvement in renal hemodynamics after aminophyllin, even the augmented mercurial diuretic response was notably less than that of a typically responsive patient without aminophyllin. In these patients and B. G., who had virtually no diuretic response even after aminophyllin, serum sodium and chloride concentrations were significantly reduced (Table II).

Therefore, both renal hemodynamic functions and serum electrolyte concentrations were increased in subsequent studies on mercurial resistant subjects by infusions of 5 per cent sodium chloride, often with sufficient molar sodium lactate added to maintain the normal serum Na/Cl ratio. In Table IV are presented observations made on patient E. S. six weeks after the previous study. During the control periods, his filtration rate, serum electrolyte concentrations, filtered electrolyte loads, and electrolyte excretion rates were still very much reduced. At the end of the 120-minute period during which 450 mEq. of 4.5 per cent sodium chloride solution were administered, there was no significant change in sodium and chloride excretion despite the increases in glomerular filtration rate, serum electrolyte concentrations, and filtered electrolytes. Then, the concentrated salt infusion was replaced by the previous mannitol—PAH infusion and 2 ml. of Mercuzanthin were administered intravenously. Seventy minutes later, when the filtered electrolyte loads were still greatly increased, there was maximal mercurial diuresis with urinary sodium and chloride excretions of 1.22 and 1.43 mEq./min., respectively, and a urine volume of 12.5 ml./min. After the peak diuresis, as the filtration rate and filtered electrolyte load began to decline, excretion

of sodium, chloride, and water decreased too, despite maintenance of the serum sodium level.

Such intravenous administration of a concentrated salt solution for two hours prior to mercurial administration conceivably might have influenced the apparent natriuretic and diuretic response to the mercurial by affecting some mechanisms for base conservation, thereby independently decreasing tubular reabsorption of sodium. However, as demonstrated by the data on patient E. S. (Table IV), there usually is no significant increase in electrolyte excretion during the early periods of concentrated salt infusion in patients in congestive failure without organic renal disease, maintained on low salt diets (1, 10). Therefore, experiments were performed in which the filtered loads of sodium and chloride were increased acutely by the injection of concentrated sodium solutions 60 to 90 minutes *after* giving the mercurial, when maximal mercurial depression of tubular activity generally has been achieved. For example, patient G. R., despite a moderate reduction in filtration rate and serum electrolyte concentrations, exhibited an adequate diuretic response to a 2 ml. intravenous injection of Mercuzanthin (Table V). As the effect of the mercurial waned and the filtration rate declined, urinary excretion of electrolyte and water decreased. At this point, when a balanced mixture of 5 per cent sodium chloride and molar sodium lactate was infused, there were prompt increases in renal plasma flow, filtration rate, serum electrolyte levels, and a simultaneous rise in urinary Na and Cl excretions, which finally were 50 per cent greater than following the mercurial alone. This urinary reflection of the improved renal hemodynamics and increased load of filtered electrolyte occurred much earlier than the usual renal response to acutely induced hypernatremia.

Similar results were obtained in patient A. R. whose diuretic response, 60 to 95 minutes after a mercurial, was only moderate (Table IV) until a 5 per cent sodium chloride infusion was given at a rate of 5 ml./min. During this infusion, which was associated with some increase in filtration rate, a greater increase in renal plasma flow, and a 15 mEq./l. increase in serum sodium concentration, there was a progressive rise in urinary sodium excretion to a value more than three times the previous post-mercurial maximum. However, in this patient, who had arteriosclerotic heart disease and possibly organic renal pathology, the continued increase may represent, in part, a secondary but independent effect of the concentrated salt infusion on urinary electrolyte excretion.

The same procedure was attempted in patient

TABLE IV

Effect of concentrated sodium infusions on the diuretic response of cardiac patients to Mercuzanthin

Patient	Period	Time	R.P.F.	G.F.R.	Serum Na	Serum Cl	Na filt.	Na excr.	Cl filt.	Cl excr.	Urine vol.
		min.	*ml./min.*	*ml./min.*	*mEq./l.*	*mEq./l.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*ml./min.*
E. S.	Control	0–53	194	55	123	89	6.74	0.002	5.32	0.004	1.97
	5% NaCl*	124–140	192	62	139	107	8.60	0.006	7.32	0.018	1.25
	Mercuz.†	194–204	194	82	136	107	11.1	1.13	9.74	1.44	12.1
		204–214	190	84	135	104	11.4	1.22	9.71	1.43	12.5
		214–227	187	75	135	103	10.2	0.998	8.55	1.29	11.4
		227–237	195	69	135	103	9.33	0.930	7.85	1.23	10.7
		237–246	188	67	135	102	9.07	0.854	7.56	1.14	10.3
		246–259	176	65	135	101	8.74	0.725	7.30	0.946	8.88
A. R.	Control	0–48	195	62	126	95	7.89	0.059	6.55	0.141	4.80
	Mercuz.‡	138–150	191	54	125	95	7.28	0.445	5.70	0.710	8.89
	Conc. Na Inf.§	178–188	267	59	137	102	8.08	1.19	6.76	1.53	14.1
		188–198	264	62	138	103	8.50	1.41	7.05	1.72	15.5
		198–208	266	62	140	106	8.73	1.60	7.34	1.93	16.5

* 5% NaCl infused I.V. at 4.5 ml./min. from minutes 53 to 173.
† Mercuzanthin 2 ml. I.V. at minutes 145–146.
‡ Mercuzanthin 2 ml. I.V. at minutes 55–57.
§ 5% NaCl (550 ml.) + Molar Na Lactate (80 cc.) infused I.V. at 5 ml./min. from minutes 161 to 208.

TABLE V

Immediate effect of concentrated sodium infusion on mercurial diuresis in chronic congestive failure

Patient	Period	R.P.F.	G.F.R.	Serum Na	Serum Cl	Na filt.	Na excr.	Cl filt.	Cl excr.	Urine vol.
		ml./min.	*ml./min.*	*mEq./l.*	*mEq./l.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*ml./min.*
B. G.	Control	62	21.8	114	82	2.48	0.001	1.79	0.000	.09
	Mercuz.	57	22.9	111	82	2.56	0.003	1.88	0.000	1.06
	5% NaCl*	81	24.8	112	84	2.78	0.007	2.09	0.000	1.20
		97	27.3	115	86	3.13	0.040	2.60	0.192	2.56
		111	29.5	119	88	3.50	0.115	2.88	0.307	3.64
		111	29.5	122	92	3.62	0.204	3.02	0.410	4.57
		110	29.6	125	94	3.70	0.263	3.10	0.467	4.88
	Stop Inf.	113	30.3	126	95	3.83	0.309	3.20	0.528	5.18
		99	25.8	126	93	3.26	0.208	2.67	0.384	3.97
		100	24.5	125	91	3.06	0.121	2.47	0.292	3.18
G. R.	Control	142	56.1	129	100	7.30	0.005	6.31	0.016	5.10
	Thiomerin	181	69.1	129	100	8.98	0.020	7.74	0.098	10.3
		166	67.2	129	101	8.76	0.021	7.53	0.087	8.81
		186	67.9	130	101	8.89	0.428	7.61	0.595	12.9
		175	65.7	130	101	8.65	0.823	7.46	1.08	14.5
		167	65.4	130	101	8.62	0.817	7.42	1.08	13.0
		154	61.4	130	101	8.10	0.723	7.00	0.995	11.0
	5% NaCl†	170	61.3	134	103	8.27	0.805	7.07	1.10	11.1
		206	66.3	138	105	9.24	1.11	7.85	1.37	11.8
		219	71.3	139	105	9.99	1.25	8.42	1.50	13.4

* 5% NaCl I.V. at 3 ml./min.
† 5% NaCl I.V. at 6 ml./min.

B. G., who three weeks previously had exhibited little potentiation of mercurial diuresis after aminophyllin injection. In the interim, her cardiovascular status had rapidly deteriorated. Her filtration rate and renal plasma flow were about 60 per cent lower than before and her serum electrolyte concentrations were still very reduced (Table V). Again, a mercurial alone had no effect on urinary electrolyte excretions. However, two hours after injecting the mercurial, when 5 per cent saline solution was infused intravenously at 3 ml./min., sodium and chloride excretion immediately rose as renal plasma flow, filtration rate, and serum electrolyte concentrations increased. Urinary electrolyte excretion was greatest when the filtered sodium and chloride loads were maximal, but fell sharply and rapidly as filtration rate decreased after the concentrated salt infusion was stopped, despite the persistance of the increased renal plasma flow and serum electrolyte levels. However, with the severely impaired cardiovascular renal hemodynamics, the electrolyte diuresis did not approach that observed during a good mercurial response. It should be noted that when the concentrated salt infusion was given after the mercurial, good diuresis occurred at a filtered sodium load of 3.83 mEq./min., whereas little diuresis occurred at a sodium load of 8.29 mEq./min. after a mercurial followed by aminophyllin (Table II). Apparently, a hypertonic saline infusion, in addition to increasing filtered load, affects still other mechanisms influencing mercurial diuresis.

The influence of a more rapid rate of infusion of concentrated salt solution was tested in patient M. M., who had the lowest serum electrolytes and most severely impaired renal hemodynamics encountered in our large series of cardiacs without renal disease (Table VI). Ninety-six minutes after intravenous injection of 2 ml. of Mercuzanthin, which had little diuretic effect, a concentrated sodium chloride-sodium lactate infusion was given at a rate of 8 ml./min. for the first ten minutes and, then, at a rate of 4 ml./min. until the end of the study. In the first fourteen-minute period after starting this infusion, there were marked increases in urinary electrolyte and water excretions, which persisted as the improvement in renal hemodynamics, serum electrolyte levels, and filtered electrolyte loads was maintained. After 40 min-

TABLE VI

Augmentation of mercurial diuresis by administration of concentrated sodium infusion and aminophyllin to mercurial-resistant patient M. M.

Period	Time	R.P.F.	G.F.R.	Serum Na	Serum Cl	Na filt.	Na excr.	Cl filt.	Cl excr.	Urine vol.
	min.	*ml./min.*	*ml./min.*	*mEq./l.*	*mEq./l.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*ml./min.*
Control	0–70	54.4	21.3	107	72.4	2.36	0.004	1.70	0.015	1.66
Mercuz.*	149–167	57.6	20.8	103	72.9	2.13	0.014	1.66	0.042	2.62
Conc. Na†	167–180	86.4	29.1	113	79.4	3.26	0.232	2.54	0.276	6.62
	180–192	83.0	26.3	117	81.5	3.06	0.219	2.36	0.294	5.59
	192–205	83.5	26.9	117	81.9	3.02	0.215	2.38	0.298	5.57
	205–216	87.5	27.3	116	80.7	3.13	0.231	2.42	0.318	5.36
Aminoph.‡	216–225	132.0	36.1	114	79.5	4.10	0.806	3.21	0.817	12.6
	225–233	94.0	34.5	113	78.9	3.86	0.639	2.99	0.642	10.7
	233–242	88.0	31.2	112	78.2	3.47	0.441	2.68	0.506	8.94

* Mercuzanthin 2 ml. at minutes = 70.

† 5% NaCl (430 ml.) + Molar Na Lactate (120 ml.) inf. I.V. at 8 ml./min. from minutes = 167–177, and at 4 ml./min. from minutes = 177–242.

‡ 0.50 gm. I.V. from minutes = 211–216.

utes, 0.5 gm. of aminophyllin were given intravenously. With the additional increases in renal plasma flow, filtration rate, and filtered electrolyte loads, there was a further prompt and marked increase in excretion of sodium, chloride and water followed by a decline during the next two periods as filtration rate and renal plasma flow began to fall. At peak diuresis, urine flow was 12.6 ml./min., and sodium and chloride excretions were 0.806 and 0.817 mEq./min., respectively.

DISCUSSION

The failure of cardiac patients to respond to mercurial diuretics probably is conditioned by many of the factors influencing salt and water retention in congestive failure. That one of these markedly impaired renal hemodynamics can affect response to these agents is clearly demonstrated by the present data. Thus, if filtration is severely depressed, the load of electrolyte delivered to otherwise normal tubules may be so reduced that there is a disproportionately much greater decrease in sodium and chloride excretion. As a result, despite the usual tubular inhibition, the reabsorptive capacity of the tubules may not be exceeded and little additional electrolyte or water will be excreted after a mercurial. In this series of mercurial-resistant patients, acute increases in renal plasma flow and filtration rate, experimentally induced after administering mercurials, were associated with significant augmentation of diuresis. When the serum electrolyte concentrations, and therefore the filtered loads of sodium and associated anions, were further elevated by giving hypertonic infusions of sodium salts, even greater immediate response to mercurials was achieved. Our preliminary report of this implied relationship between filtered electrolyte load and response to mercurial diuretics has been confirmed by the observations of Pitts and Duggan (11) on dogs and normal men.

Thus, the previous diuretic unresponsiveness of these patients was not due to tubular resistance to mercury. That renal tubular functions other than electrolyte reabsorption also are affected to the usual degree by mercurials in such a patient (M. L.) is illustrated by the 60 per cent decrease in Tm_{PAH} without any change in water or chloride output (Table VII), produced by an injection of 2 ml. of Thiomerin.

TABLE VII

Effect of Thiomerin on Tm_{PAH} *and chloride excretion of mercurial-resistant patient in congestive failure*

Period	G.F.R.	Plasma PAH	T_{mPAH}	Chloride excretion	Urine volume
	ml./min.	*mgm./ml.*	*mgm./min.*	*mEq./min.*	*ml./min.*
Control	47.1	0.600	79.6	.002	3.05
Merc.	32.0	0.923	32.0	.026	3.80

TABLE VIII

Effect of pre-treatment with desoxycorticosterone acetate on mercurial diuresis in patient G. R.†*

Period	R.P.F.	G.F.R.	Serum Na	Serum Cl	Na filt.	Na excr.	Cl filt.	Cl excr.	Urine vol.
	ml./min.	*ml./min.*	*mEq./l.*	*mEq./l.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*ml./min.*
Control	175	59.4	130	93.6	7.84	0.007	6.21	0.011	5.98
Thiomerin	164	60.5	129	93.4	7.84	0.011	6.29	0.009	7.23
	161	60.6	129	94.1	7.87	0.009	6.36	0.016	7.29
	161	59.4	130	94.9	7.73	0.011	6.28	0.023	6.63
	150	55.4	130	94.7	7.20	0.015	5.84	0.035	5.94
	147	52.1	130	94.6	6.78	0.031	5.49	0.061	5.79
	148	53.6	130	93.9	7.00	0.071	5.60	0.125	6.65
	156	54.6	130	92.7	7.11	0.143	5.64	0.229	8.25
	150	53.1	129	91.9	6.91	0.171	5.44	0.268	8.76
	154	54.2	129	91.0	7.01	0.273	5.51	0.402	10.6

* 4 Doses of DCA (5 mgmq. 6 hours).
† Cf. Table V.

It should be re-emphasized, however, that any factor promoting renal salt conservation will tend to antagonize mercurial diuretics. Thus, if tubular reabsorption of electrolyte is significantly enhanced by any mechanism, mercurial inhibition of reabsorption in one part of the tubule may produce much less diuresis because of greater transfer of electrolyte and water back into the blood, perhaps at some other tubular site. The inhibition of mercurial diuresis by a humoral factor, for example, is illustrated by a subsequent study on G. R. who was given the same dose of Thiomerin before and after receiving four 5 mgm. doses of desoxycorticosterone acetate (DCA) at 6-hour intervals in the previous 24-hour period (Table VIII). Before receiving DCA, she had achieved maximal diuresis with a urine volume of 14.5 ml./min. and sodium and chloride excretion rates of 0.823 and 1.08 mEq./min., respectively, within an hour (Table V). Eight days later, after receiving the desoxycorticosterone acetate, her body weight, clinical condition, and serum sodium levels were unchanged and her serum chloride level, filtration rate, and renal plasma flow were slightly lower. Now, one hour after receiving the mercurial, her sodium and chloride excretion rates were only 0.0107 and 0.0234 mEq./min., respectively. In the next hour, electrolyte and water excretions gradually increased, but never equalled the levels reached before DCA administration. Others have observed that, by administration of DCA or ACTH (12), inhibition of mercurial activity may be produced, which varies in degree depending upon the duration and intensity of treatment and other factors.

Such humoral influences on tubular reabsorption may have contributed to the gradually developing failure of response to mercurials observed in patient J. B. during the severe salt restriction of the rice diet, which may lead to increased adrenal cortical activity as well as to altered renal hemodynamics (8). Yet the latter must also have played a role in view of the augmentation of mercurial diuresis observed when filtration rate and renal flow were increased by aminophyllin.

The activation of other sodium-conserving mechanisms in congestive failure may explain certain other well documented aspects of mercurial diuresis. A number of investigators (1, 2, 13) have confirmed the early observations of Blumgart and his co-workers (14, 15) that during mercurial diuresis not only may relatively more chloride than sodium frequently be excreted but also potassium excretion may be increased in patients exhibiting sodium retention. The explanation for these phenomena may be the same: namely, that the kidney can actively conserve sodium without chloride by tubular transport of potassium, ammonia, and hydrogen. When the body responds to a stimulus leading to sodium retention, as in congestive failure, these ion exchange mechanisms probably are also stimulated. As a consequence, sodium that has escaped reabsorption because of mercurial depression of one tubular activity may be actively exchanged for other cations by distal tubular processes which may be less

TABLE IX

Effect of pre-treatment with desoxycorticosterone acetate (DCA) on mercurial diuresis in patient G. Re.*

	Period	Time	Urine vol.	Urinary electrolyte excr.			Serum electrolytes		
				Na	Cl	K	Na	Cl	K
		min.	*ml./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./l.*	*mEq./l.*	*mEq./l.*
Before DCA	Control	100–0	1.70	0.005	0.004	0.030	138	102	5.40
	Thiomerin†	0–30	1.90	0.012	0.021	0.024			
		30–60	3.17	0.187	0.200	0.036			
		60–90	5.83	0.432	0.478	0.057			
		90–152	2.93	0.320	0.363	0.051			
		152–182	2.38	0.266	0.301	0.047			
		182–240	2.82	0.233	0.307	0.046	138	101	4.87
After DCA	Control	91–0	3.10	0.004	0.014	0.047	139	106	5.63
	Thiomerin†	0–30	3.90	0.040	0.048	0.049			
		30–60	4.14	0.217	0.281	0.060			
		60–90	5.23	0.334	0.449	0.092			
		90–150	3.22	0.218	0.332	0.106			
		150–180	2.10	0.176	0.292	0.112			
		180–240	1.52	0.099	0.196	0.092	139	105	4.50

* 20 mgm. of DCA/day for 4 days.
† 2 ml. of Thiomerin.

affected by therapeutic doses of mercurials (16). Since these ion exchange mechanisms function to conserve sodium without chloride during mercurial diuresis, there may be an increase in urinary potassium, ammonia, and titratable acidity and a greater excretion of chloride than of sodium.

This possibility is illustrated by the data obtained on G. Re. before and immediately after four daily injections of 20 mgm. of desoxycorticosterone acetate (Table IX). The more prolonged administration of DCA to this rheumatic cardiac who was maintained on a low salt diet, did not abolish the diuretic response but did lead to increased potassium diuresis and a significant reduction in the excretion of sodium relative to chloride. Before DCA treatment, the cumulative chloride, sodium, and potassium excretions in the 240 minutes after administering the mercurial were 70.4, 60.3, and 10.8 mEq., respectively. After giving DCA, the chloride, sodium and potassium excretions in the equivalent post-mercurial period were 63.8, 41.9, and 21.3, mEq., respectively.

Titratable acidity and ammonia production also have been demonstrated to increase during mercurial diuresis in cardiac patients (2, 16). Since the sum of potassium, ammonium ion and titratable acid excretions tends to equal the excess of chloride over sodium excreted during the mercurial diuresis (1, 13), it would appear that ion exchange mechanisms may be responsible for the reported discrepancy between urinary sodium and chloride as well as the increased potassium excretion. However, the primary diuretic action of the mercurial may well have been on tubular reabsorption of sodium and associated ions, as others have suggested (17).

In cardiac patients without organic renal disease, intensification of one or more of the factors promoting sodium retention in congestive failure —impaired cardiovascular-renal hemodynamics, elevated venous pressure, altered serum electrolytes, increased adrenal cortical activity, stimulation of volume receptors (18), and other variables (4, 5)—may contribute to the development of mercurial resistance. Although, in this discussion, a correlation between increased filtered electrolyte load and augmentation of mercurial diuresis by administration of aminophyllin or concentrated salt solutions has been implied, it is recognized that either procedure may have also influenced other mechanisms affecting electrolyte excretion. Aminophyllin, for example, invariably produced an even greater increase in renal plasma flow in these patients (19), a circumstance which is often associated with improved electrolyte excretion in cardiac failure (20, 21, 22). In patients in congestive failure, this drug may also produce a less persistent but significant increase in cardiac out-

put and a decrease in venous pressure (19, 23) with consequent augmentation of sodium excretion, particularly when renal tubular reabsorption has already been depressed by a mercurial or perhaps by the aminophyllin itself (24, 25). Similarly, concentrated sodium infusions, in addition to producing the increases in filtered electrolyte load, may have influenced adrenal cortical, volume or renal mechanisms regulating electrolyte excretion. As in interpreting the role of those agents which potentiate mercurial diuresis, so in analyzing mercurial resistance *per se*, it is futile to seek a single explanation applicable to all cases. In the individual patient, however, careful evaluation will often uncover the more important causal factors and thereby permit better therapy.

In the past five years, this approach to mercurial resistance has modified our treatment of patients who previously gave poor diuretic response to mercurials, even following pre-treatment with ammonium chloride. Effective mobilization of edema frequently has been achieved by the following procedure: *1*) 6 to 9 gm. of ammonium chloride are given daily in divided doses for three days; *2*) on the fourth day, 2 ml. of a mercurial are given; *3*) two hours later, when maximal mercurial depression of tubular function is usually present, from 0.25 to 0.50 gm. of aminophyllin are injected intravenously over a period of 5 to 10 minutes. After receiving the mercurial, the patients are kept in bed until diuresis has subsided, since activity, which increases salt and water retention in congestive failure (24), also will tend to diminish the effect of mercurials. In patients who have been almost totally unresponsive to mercurials, profuse diuresis with marked weight loss has resulted. However, to avoid reactions, especially in elderly subjects, the aminophyllin should be given very slowly and cautiously.

It should be emphasized that in the present studies infusions of hypertonic sodium solutions were given experimentally to improve both renal hemodynamics and serum electrolyte levels. This procedure generally is not therapeutically practical and may even be harmful except perhaps in the presence of the *acute depletion hyponatremia*, associated with vascular collapse and further impairment of renal function. Generally, the amounts of sodium required to improve diuretic response are so great that the net effect, despite better diuresis, is salt retention, thirst, increased water intake, and more edema. In *dilution hyponatremia*, hypertonic saline is contra-indicated.

SUMMARY

The ultimate failure of cardiac patients to respond to mercurial diuretics has been re-investigated as a phenomenon of renal electrolyte dysfunction in advanced heart failure.

In mercurial-resistant patients, the increased diuretic response achieved by injecting 0.48 to 0.72 gm. of theophylline ethylenediamine (aminophyllin) intravenously, 90 to 120 minutes after administering a mercurial, was much greater than that observed following aminophyllin alone, and was associated with an increase in the severely impaired renal plasma flow, filtration rate, and filtered load of electrolytes. In patients with reduced serum sodium and chloride concentrations, there was less augmentation of mercurial diuresis following aminophyllin administration. In such patients, acute increase of the serum electrolyte levels by infusion of concentrated sodium solutions intravenously, 90 to 120 minutes after giving a mercurial, resulted in an immediate increase in water, sodium, and chloride diuresis, which was much greater than the delayed diuresis following hypertonic salt infusions alone.

Although the improved response to mercurials in resistant patients following these procedures correlated well with the increase in filtered sodium and chloride loads, it is suggested that the diuretic response may have been influenced also by the concomitant changes in other factors which may affect tubular reabsorption of electrolyte. Thus, administration of desoxycorticosterone acetate in large doses for 24 hours was found to depress electrolyte diuretic response to a mercurial. Continuing such doses for four days before giving a mercurial resulted in greater decrease in sodium than chloride excretion with an equivalent increase in potassium excretion in a cardiac patient on a low salt diet. It is suggested *1*) that adrenal cortical activity may also contribute to mercurial resistance by increasing tubular reabsorption of sodium; and *2*) that the distal tubular ion exchange mechanisms for conserving sodium may be responsible for the increased potassium excretion and apparently greater chloruresis than natri-

uresis observed after mercurials in many sodium-retaining patients.

It is concluded that the failure of cardiac patients to respond to mercurial diuretics results from the influence of the many factors promoting sodium retention in congestive failure.

ACKNOWLEDGMENTS

The authors wish to thank Miss Sybil Goldat, Mrs. Maidie Ross, and Mr. George Ross for their technical assistance.

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THE TRANSPORT OF FRUCTOSE BY HUMAN PLACENTA[1]

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(Submitted for publication June 10, 1952; accepted August 1, 1952)

The presence of fructose in fetal blood was first definitely established by Bacon and Bell (1). They found, in agreement with earlier investigators (cf. Cole and Hitchcock [2]), that throughout gestation sheep fetal blood contains an appreciable amount of a ketohexose which they characterized as fructose by its optical rotation and by the preparation of derivatives. Subsequent studies (3) by other investigators revealed a difference in the fructose concentrations of maternal and fetal blood, and demonstrated that sheep placenta actively transfers fructose into the fetal circulation; however, no similar evidence for an active transfer of glucose was obtained. In the human being, Orr (4) has shown that umbilical cord blood obtained at the time of birth contains a levo-rotary reducing sugar which gives a positive Seliwanoff test. Previous studies in this laboratory (5) have shown that human placenta actively secretes glucose up to about the twelfth week of gestation after which this ability is usually lost. In the course of studies of the biochemistry of human reproduction, the amount and source of fructose in human fetal blood has been investigated. The present report describes evidence *1*) that an infant's blood at the time of birth contains about 4 mgm. per cent of fructose, while at the same time the maternal blood contains less; and *2*) that the full-term human placenta can secrete fructose.

METHODS

Analytical. Blood was obtained from the mother and from the umbilical cord immediately following delivery of the placenta, using the usual mixture of ammonium and potassium oxalate as an anticoagulant. For fructose analyses, blood proteins were precipitated by adding an equal volume of 20 per cent cold trichloracetic acid to the blood. For the determination of total reducing sugars ("glucose") the method of Nelson was used (6).

Using the resorcinol method of Roe as modified by Higashi and Peters (7), control experiments with pure standard solutions of glucose and fructose gave quantitative, reproducible results. With this procedure glucose gives only 0.9 per cent as much color as an equivalent amount of fructose. A few fructose analyses were also done by the diphenylamine method described by Kendrick (8). In this procedure glucose gives 3 per cent as much color as fructose does, so that a larger correction is required for the amount of glucose present and reacting as fructose. In addition, the diphenylamine method gave much higher fructose values than those obtained concurrently by the resorcinol method. For these reasons the resorcinol method was used in the majority of the analyses.

In vitro studies. Placenta slices weighing about 200 mgm. were incubated in Warburg vessels at 37.5° C. with an atmosphere of 100 per cent oxygen. The medium was a modified Krebs-Ringer phosphate buffer containing 90 mM/L. Na^+, 50 mM/L. K^+ and 10 mM/L. Mg^{++} with 100 mgm. per cent glucose added as a carbohydrate substrate. The pH of the buffer was 6.9 at the beginning of the experiment and 6.9 ± 0.1 at the end of the two-hour incubation.

Analyses of the medium for total reducing sugar and fructose were performed as described above for blood.

Chromatographic methods. The trichloracetic acid filtrates of cord blood were extracted with ether to remove the organic acid and concentrated *in vacuo* to a suitable volume. The residues were placed on Whatman No. 1 filter paper and descending chromatograms were developed using 3:3:1 ethyl acetate, water and acetic acid (9). Aniline oxalate was used as a color reagent for locating the sugars on the chromatograms.

RESULTS

The blood analyses are summarized in Table I. In accordance with the results of other investigators (cf. Smith [10]), the fetal blood sugar content was found to be significantly lower than the maternal (S.E. of the mean difference = 2.8, P less than 0.01). However, the fructose level of the cord blood was 45 per cent *higher* than that of the maternal blood (S. E. of the mean of the differences 0.2; P less than 0.01). Thus the term human placenta can apparently transfer fructose by some active process into the fetal blood stream.

[1] This investigation has been aided by grants from the Charles A. King and Marjorie King Fund and from Winthrop-Stearns, Inc.

TABLE I

Fructose and glucose content of human fetal and maternal blood

	Fructose (resorcinol method)	Fructose (Diphenyl-amine method)	Glucose (Nelson)
Maternal blood	2.9*±0.1 (18)†	8.6 (4)	88±6 (9)
Cord blood	4.2 ±0.3 (20)	10.4 (4)	74±7 (9)

* All values as mean ± the standard error in mgm./100 ml. blood.

† The number in parentheses indicates the number of experiments.

Incubation *in vitro* demonstrated that placenta does secrete fructose, as summarized in Table II. Although the amounts of fructose secreted are not large, it is clear that placenta possesses the enzyme system, presumably a fructose-6-phosphatase, required for this process.

An alternative hypothesis would be that the higher level of fructose in the fetal blood is brought about by the secretion of fructose by the liver of the fetus. To test this, incubation experiments were performed using rat liver slices instead of placenta slices in the system described above. These experiments showed that no fructose is secreted by rat liver and render unlikely the hypothesis that human fetal liver can secrete fructose.[2]

It is well known that the resorcinol and diphenylamine procedures are not entirely specific for fructose so other evidence of the chemical identity of the substance reacting as fructose was sought. The absorption spectra of the reaction products of resorcinol with the unknown sugar and with pure fructose were compared (Figure 1). It is clear that genuine fructose and the material measured in blood as fructose give identical products with resorcinol as measured by their spectral absorption characteristics. More direct evidence

[2] These incubations were performed in cooperation with Drs. A. Renold and A. B. Hastings.

TABLE II

Glucose consumption and fructose production by human placenta slices incubated in vitro

No. of vessels	30 (7 placentas)
Oxygen consumption	1.7±0.3* ml./gm. dry wt./hr.
Glucose disappearance	8.0±0.4 micromoles/gm. wet wt./hr.
Fructose appearance	0.17±0.03 micromoles/gm. wet wt./hr.

* Values ± S.E.

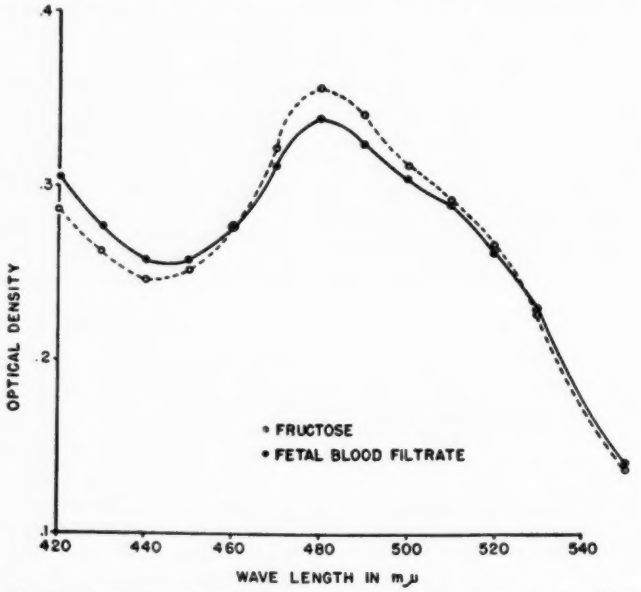

FIG. 1. ABSORPTION SPECTRA OF RESORCINOL REACTION PRODUCT WITH FETAL BLOOD FILTRATE AND WITH PURE FRUCTOSE (BECKMAN SPECTROPHOTOMETER, MODEL D.U.)

was obtained by paper chromatography. Although the salts and other materials present in the blood filtrates caused rather erratic behavior of the samples on chromatography, a clear separation of the aniline oxalate-reacting material into two components was obtained. Both these components moved more rapidly than did pure glucose and fructose under the same conditions. However, when pure fructose was added to a blood filtrate (adult fasting male) that showed no fructose spot on chromatography before addition of the sugar, the added fructose moved at a rate identical to that of the more rapidly moving component of fetal blood filtrates. This is good evidence that the umbilical cord blood does contain appreciable amounts of fructose. The isolation of fructose and its identification by classical chemical methods were not attempted, since the small amount present would have required extensive manipulation with considerable opportunity for the formation of fructose by isomerization from the glucose present in much larger amounts.

DISCUSSION

The resorcinol method for fructose analysis is not completely specific, but it is possible to make a correction for the major part of the error, which is caused by glucose. After this correction has been applied, values of 1–2 mgm./100 ml. were found for blood from fasting adult males and nonpregnant females. Higher values up to 6 mgm./100 ml. blood were found in non-fasting adults. At the time of birth, maternal blood contains about 2 to 4 mgm./100 ml. of fructose while the infant's blood contains more.

Studies on sheep (2) have shown that the fetal blood fructose concentration is quite high during the early months of gestation, and possibly there is a similar high fructose level in human fetal blood early in pregnancy. However, it is difficult to obtain sufficient human fetal blood earlier in pregnancy for analysis and this has prevented experimental verification of the hypothesis. The diabetic adult human seemingly utilizes parenteral fructose with considerably less difficulty than glucose (11). Studies of human fetal tissues have revealed no striking limitation in their ability to utilize glucose (12), but perhaps a double supply of carbohydrate, entering the cells by the action of two different enzyme systems, is provided for the fetus as a safety measure to reduce the hazards of its early intra-uterine life.

SUMMARY

1. Fructose has been identified in human umbilical cord blood on the basis of the absorption spectrum of its resorcinol reaction product and by its behavior on filter paper partition chromatography.

2. Human umbilical cord blood at the time of birth contains about 45 per cent more fructose than does maternal blood at the same time.

3. Human placenta at term is capable of secreting small but significant amounts of fructose when incubated *in vitro*.

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SOME HORMONAL ASPECTS OF WATER EXCRETION IN MAN [1]

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(Submitted for publication February 23, 1952; accepted August 1, 1952)

In 1918 von Hann (1) suggested, as a result of her post mortem studies in man, that destruction of the posterior pituitary caused diabetes insipidus only if significant portions of the anterior pituitary gland remained intact. This observation has been supported by other workers who noted that in experimental diabetes insipidus, destruction of the anterior pituitary resulted in decrease in the polyuria and polydipsia (2–7).

In 1935 Mahoney and Sheehan (8) showed that experimental diabetes insipidus in the dog can be ameliorated by thyroidectomy and the polyuria can be reestablished by thyroid feeding. Fisher, Ingram and Ranson (9) confirmed this result in experimental diabetes insipidus as have others (6, 7, 10–14). Similar relationships for adrenal cortical activity to the diabetes insipidus state have been claimed (15, 16) and much has been said regarding the diuretic action of adrenal hormones (16, 17). It should be stressed that all this work was done on animals in which the degree of polyuria and polydipsia was the sole criterion of the diabetes insipidus state.

This study was done on a patient who had, in addition to well documented neurohypophyseal insufficiency, evidence of some concomitant anterior pituitary failure. The purpose of this paper is to reemphasize the physiological defect in diabetes insipidus; and to study its relationship to solute loading, thyroid, adrenal, and anterior pituitary function. The findings clearly show that polyuria and polydipsia are not necessary accompaniments of insufficiency of the supraopticohypophyseal system and suggest that the anterior pituitary, adrenals, and thyroid do not produce hormones which possess direct diuretic actions.

METHODS

Balance studies were conducted on the Metabolism Ward of the Massachusetts General Hospital (18). Urine collections were made in 24-hour periods. All bloods were drawn in the fasting state before breakfast except the second sample taken during each dehydration period, which was taken just prior to lunch.

Serum sodium concentrations were done in duplicate by the method of Butler and Tuthill (19). Serum and urine total solute concentrations were determined by the freezing point method utilizing an electrical resistance thermometer apparatus described by Crawford and his associates (20). The apparatus was standardized with sodium chloride solutions of known concentration. The assumption was made, for convenience of expression, that the sodium chloride was totally dissociated and the results were expressed as milliosmols per liter. More correctly, total solute concentration should be expressed as the concentration in millimols of sodium chloride which produces an equivalent depression of the freezing point. This expression is simply obtained by dividing the reported concentration in milliosmols by 2. All sera were measured without dilution while urines were diluted, only when necessary, into the range of 100 to 300 mOsm. per liter. This dilution of the urine, by changing the activity coefficients of certain solutes, introduces a slight error which does not affect our interpretations.

Serum and urine potassium and urine sodium were measured with an internal standard flame photometer (21). Serum and urine chlorides were done by the method of Wilson and Ball (22); serum carbon dioxide content, by the method of Van Slyke and Neill (23); serum and urine non-protein nitrogen, by the method of Folin (24); basal metabolic rate, by the closed circuit method of Benedict and Roth (25); renal clearances according to the procedures outlined by Goldring and Chasis (26).

CASE HISTORY

Mrs. A. J. (M.G.H. 606322) was a 26-year old white woman first seen by Dr. Fuller Albright in January, 1948, at which time she complained of polyuria and polydipsia of two and one half years duration. Symptoms had started during her only pregnancy which terminated in a normal delivery. Urine volumes of 8 liters per day were

[1] Supported in part by a Grant-in-Aid from the American Heart Association.

[2] National Research Council Fellow in Medical Science 1949–1951, currently Howard R. Hughes Fellow in Medicine.

[3] National Institutes of Health Post-Doctorate Research Fellow in Medicine.

noted. Amenorrhea and weight gain of 20 pounds were present over the past 10 months. Her history was otherwise negative and her physical examination was entirely negative except for very slight obesity.

Laboratory studies revealed persistently dilute urines having specific gravities of 1.003 to 1.005 but showing no other abnormalities. Urine culture was negative. Serum sodium, chloride, cholesterol, calcium, phosphorous, nonprotein nitrogen and blood sugar were normal. The urine follicle-stimulating-hormone assay (27) was weakly positive for 6 mouse units per 24 hours at this time. The hypertonic saline test (28) was positive for neurohypophyseal insufficiency and her urine concentration showed a normal response to pitressin. X-ray examination of the bones revealed a rarified area in the left parietal region measuring 2.2 by 2.5 cm. Biopsy of this area was unsuccessful. A presumptive diagnosis of eosinophilic granuloma as the underlying cause of the diabetes insipidus was made. She completed a first course of x-ray therapy to the pituitary region of 1000γ on June 29, 1948. She was placed on a weight reduction diet and given posterior pituitary extract by nasal insufflation for control of the polyuria.

During the remainder of 1948 and most of 1949 she got along well though she had no menses. She reduced her weight to 110 pounds. However, in December, 1949 she was readmitted to the Hospital with the complaints of anorexia, loss of thirst and of polyuria, and marked drowsiness for the preceding six weeks. Examination revealed only a very lethargic patient with a poor memory for recent events who frequently fell asleep during meals or visits. Posterior pituitary extract was withdrawn without recurrence of polyuria or polydipsia. Urine volumes as low as 800 ml. in 24 hours were recorded in the Hospital when no posterior pituitary extract was given, volumes certainly not likely to suggest posterior pituitary insufficiency! A second course of x-ray therapy of 1000γ to the pituitary region was completed in February, 1950.

This spontaneous disappearance of polyuria and polydipsia was reminiscent of the amelioration of experimental diabetes insipidus in animals when the anterior pituitary is destroyed. Hence a survey of the patient's endocrine status was made to evaluate anterior pituitary function with the following results:

1. Amenorrhea with urine Follicle Stimulating Hormone (27) negative for 3 mouse units on April 17, 1950.

2. Loss of axillary and pubic hair in the past few months with urinary 17-ketosteroid excretions (29) of 0.0 mgms. per 24 hours on February 14, 1950, 0.4 mgm. per 24 hours on March 10, 1950 and 1.6 mgm. per 24 hours on September 24, 1950.

3. Epinephrine-eosinophile response (30) of −33 per cent on March 27, 1950.

4. Coldness and dryness of the skin and "myxedematous" reflexes (slow relaxation phase) the past year with failure to perspire the past summer. Serum protein-bound iodine (31) was 1.9 gamma per cent on September 25, 1950 and 2.6 gamma per cent on November 2, 1950. The basal metabolic rate was found to be −29, −33 and −30 per cent on November 1, 2, and 3 respectively.

5. The insulin tolerance test (32) on two occasions was found to be essentially normal.

Hence the preponderance of evidence indicates that the patient had partial, but not complete, anterior pituitary failure as well as the previously known deficiency of neurohypophyseal function.

RESULTS

A. Urine concentration

The disappearance of polyuria in association with evidence of anterior pituitary failure in this patient with neurohypophyseal insufficiency, brings up the question of what has happened to the renal concentrating ability. Has the polyuria disappeared because of a return of concentrating ability by the kidney when anterior pituitary as well as neurohypophyseal influences are withdrawn?

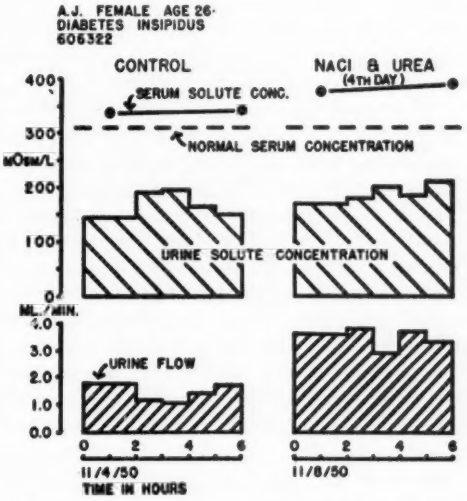

FIG. 1. THE EFFECT ON URINE AND SERUM TOTAL SOLUTE CONCENTRATION OF TWO 14-HOUR PERIODS OF WATER DEPRIVATION

In spite of marked dehydration, indicated by the high serum total solute concentration, the urine remains very dilute, distinctly hypotonic to the serum. The serum Na concentration rose to 173 mEq./L. in the second dehydration period. This inability to elaborate a concentrated urine, in spite of an increased effective total solute concentration of the extracellular fluid, is the physiological defect which characterizes neurohypophyseal insufficiency.

In Figure 1 the results on urine and serum total solute concentrations of two 14-hour periods of water deprivation are shown. Note that in spite of marked dehydration, which is indicated by the high serum total solute concentrations, the urine remains dilute, distinctly hypotonic to the serum. This is contrary to what is seen in the normal human being during water deprivation when a much smaller increase in serum total solute concentration is associated with a very concentrated urine 1000 to 1200 mOsm. per liter (33). This inability to concentrate the urine in the presence of an increased effective osmotic pressure of the serum is the characteristic defect of neurohypophyseal failure (34). Hence this defect was not ameliorated by anterior pituitary insufficiency.

B. Why did the polyuria disappear?

If the patient still exhibits a failure of renal concentrating ability, the explanation for the disappearance of her previous polyuria must be a low total solute excretion. A large volume of urine was not necessary to excrete a small total solute load even though the urine remained dilute.

While receiving no medications the subject was placed on a diet of her own choosing. Her average 24-hour urine volume was 2844 cc. and total solute excretion was 388 mOsm. per 24 hours. This is to be contrasted with the corresponding data from eight normal controls who excreted an average of 866 mOsm. per 24 hours. If the subject with her inability to concentrate the urine had chosen a diet that yielded an excretory load equal to the average figure for the controls, she would have required a daily urine volume of some 6500 cc. for its excretion. Such a urine volume would have been compatible with clinically recognizable diabetes insipidus. Thus the disappearance of polyuria in this patient reflected the decreased excretory solute load.

C. Effects of solute loading

If this concept that the disappearance of polyuria indicated a reduction in excretory solute load were correct, then giving a solute load should cause return of the polyuria. To test this, the patient was placed on a fixed diet of her own choosing but an *ad libitum* water intake. After four control days, the subject was given 10 gm. of sodium chloride and 15 gm. of urea orally daily in six divided dosages for four days (Figure 2). If this were all absorbed it would have added an estimated 595 mOsm. per day to the urinary excretory load. As seen in Figure 2 this increase in urinary solute excretion was accomplished with only a slight increase in urine solute concentration, hence urine volume rose from less than three liters daily during the control to slightly over six liters in one day of the after period. These results indicate that the polyuria, which has become generally accepted as the primary clinical requisite for the diagnosis of neurohypophyseal failure, is dependent upon the quantity of urinary solute and not alone upon the inability to concentrate the urine.

D. The effects of hormone administration on water excretion

Thus far it seemed that the loss of polyuria associated with anterior pituitary insufficiency could be explained without having to consider possible direct diuretic effects of the endocrines. If this subject could be said to have total absence of anterior pituitary function, then the demonstration that the physiological defect of neurohypophyseal insufficiency persisted would be sufficient proof that the anterior pituitary and its subsidiary glands do not produce diuretic hormones which directly affect renal concentrating ability. If the renal concentrating defect of neurohypophyseal insufficiency is unaltered in the absence of anterior pituitary activity then clearly the presence of the latter could not be the cause of this concentrating defect. As clinical methods do not currently afford sufficient sensitivity to establish complete absence of anterior pituitary activity, it was felt that administration of hormones for which direct diuretic effects have been claimed might be of interest. With the subject on a fixed diet affording a constant daily solute load (within the limits of the changes in protoplasmic content that might occur during the study), the only way a hormone could have a direct diuretic action would be to reduce still further the concentrating ability of the subject's kidneys so that a larger urine volume would be required to excrete the fixed excretory load.

Thus thyroxine, 0.75 mgm. daily, was given intravenously resulting in an increase in BMR

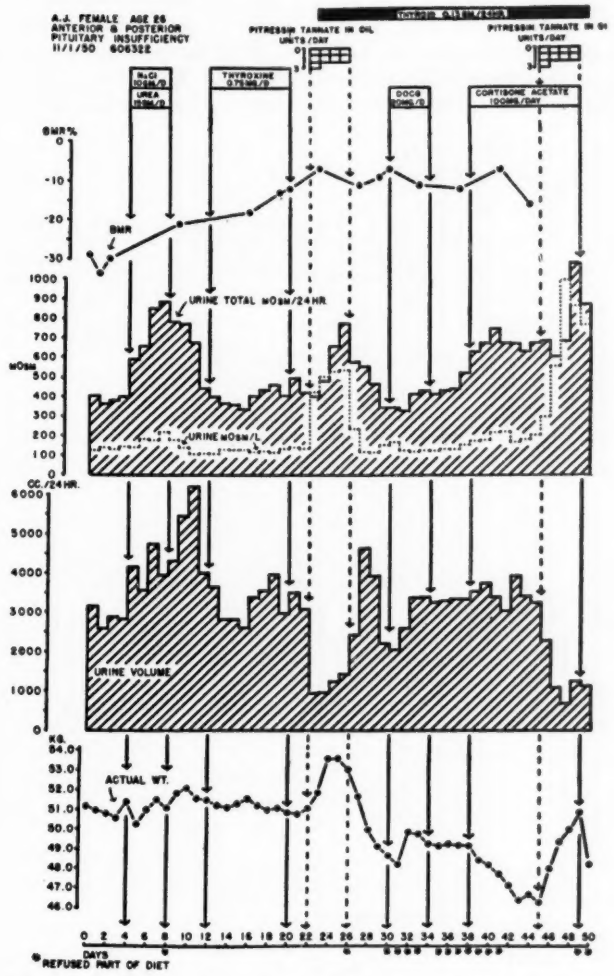

FIG. 2. THE EFECT OF SOLUTE LOADING, INTRAVENOUS THYROXINE, PITRESSIN, DESOXYCORTICOSTERONE GLUCOSIDE, CORTISONE AND CORTISONE WITH PITRESSIN ON BMR, URINE TOTAL SOLUTE EXCRETION PER 24 HOURS, URINE TOTAL SOLUTE CONCENTRATION, URINE VOLUME AND BODY WEIGHT WHILE ON A FIXED DIET BUT AN AD LIB. WATER INTAKE

Solute loading produced an increase in excretion of solute accomplished with only a slight increase in urine concentration and hence polyuria returned. Thyroxine, desoxycorticosterone and cortisone caused no decrease in urine concentration or increase in urine volume as compared with the control days. Pitressin, both with and without cortisone, resulted in antidiuresis, concentrated urine, weight gain, and an increased total solute excretion reflecting the chloruresis and natriuresis.

from −30 to −7 per cent with a rise in serum protein-bound iodine concentration from 2.6 to 14 gamma per cent. However there was no further dilution of the urine, and urine volumes remained at the control level (Figure 2). Hence thyroxine could not be demonstrated to have a direct diuretic action in the conditions of this study.

Next pitressin tannate in oil (Parke Davis & Co., Lot K888j) was administered intramuscularly. Two units were given initially followed by

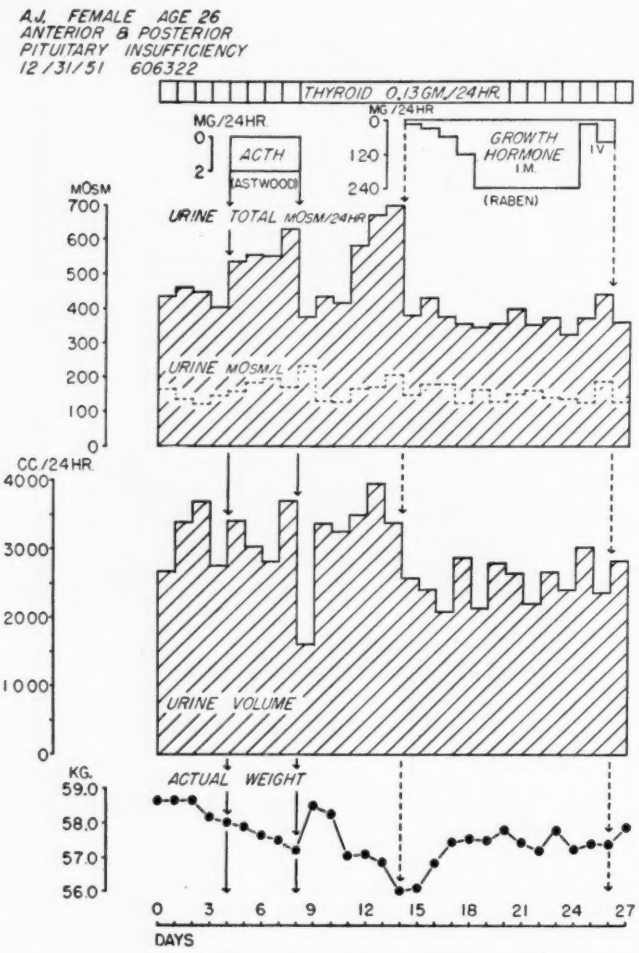

FIG. 3. THE EFFECT OF PURIFIED ACTH AND GROWTH HORMONE ON URINE TOTAL SOLUTE EXCRETION PER 24 HOURS, URINE TOTAL SOLUTE CONCENTRATION, URINE VOLUME AND BODY WEIGHT WHILE ON A FIXED DIET BUT AN AD LIB. WATER INTAKE

ACTH produced an increase in solute excretion due to an increase in nitrogen excretion (see Figure 8). The second peak in solute excretion resulted from the sodium and chloride diuresis which followed cessation of ACTH administration (see Figure 9). Neither ACTH nor growth hormone effected a dilution of the urine below control concentrations.

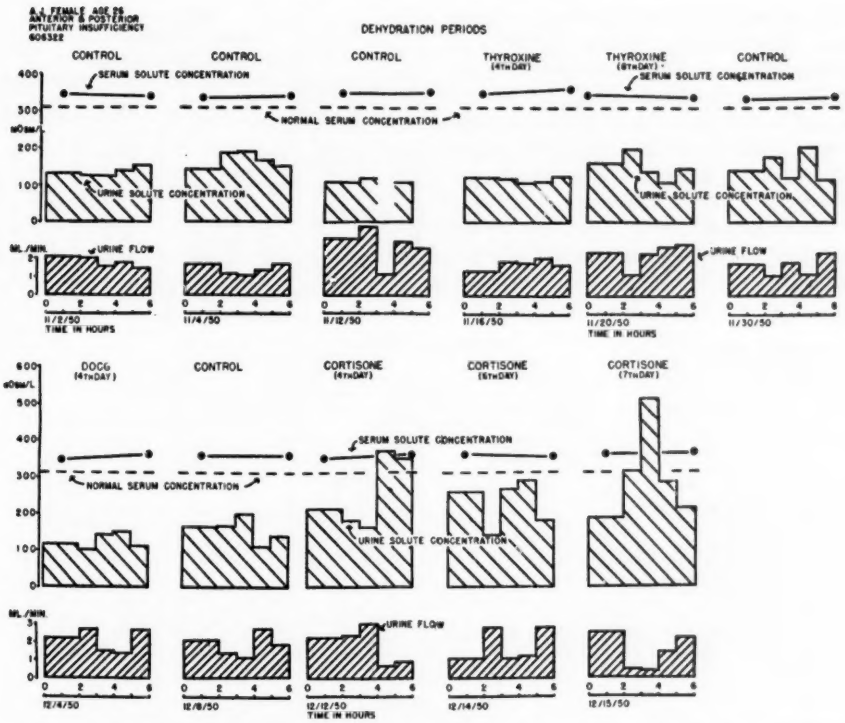

FIG. 4. THE RESULTS OF A STANDARD (14-HOUR) WATER DEPRIVATION ON SERUM TOTAL SOLUTE CONCENTRATION, URINE TOTAL SOLUTE CONCENTRATION AND URINE FLOW

Thyroxine, desoxycorticosterone and cortisone caused no significant lowering of urine concentration or increase in urine flow. Hence they failed to exhibit a diuretic effect under these conditions. "0" on the ordinate was actually the eighth hour of water deprivation.

1 unit every 12 hours for 4 days. The characteristic reduction in urine volume and increase in concentration of the urine with the small amounts of pitressin administered indicates that the subject's kidneys respond normally to antidiuretic hormone (Figure 2).

Desoxycorticosterone glucoside given in four divided dosages totalling 20 mgm. daily intramuscularly followed by cortisone acetate 25 mgm. four times daily intramuscularly failed also to exhibit diuretic effects; urine concentration did not fall and urine volumes remained at control levels [4] (Figure 2). Desiccated thyroid 0.13 gm. daily orally was administered throughout the remainder of the study following cessation of thyroxine injections to preserve a constant normal level of thyroid hormone activity and to exclude the possibility that the other hormones required a normal metabolic rate in order to manifest their diuretic action.

On a subsequent admission, but while receiving the same diet as previously, corticotrophin [5] (Ast-

[4] It should be mentioned that anorexia persisted following recovery from the water intoxication produced by pitressin—so that the patient refused part of her diet on several occasions (Figure 2). In spite of some dietary refusals during cortisone, the total solute excretion rose, due largely to catabolism of body protein with increased nitrogen excretion. This effect of cortisone together with the dietary refusals explains the weight loss during this period (Figure 2).

[5] We are indebted to Drs. E. B. Astwood and M. S. Raben for generously supplying us with their purified corticotrophin and growth hormone, respectively.

wood) and growth hormone [5] (Raben) were administered. Corticotrophin in four divided dosages intramuscularly, totalling 2.0 mgm. daily for four days, and growth hormone in divided dosages totalling up to 240 mgm. daily intramuscularly and 80 mgm. daily intravenously for a total of twelve days likewise failed to exhibit diuretic effects (Figure 3). Renal clearances with inulin and *p*-aminohippurate during this study reveal that the reduced clearances characteristic of pituitary insufficiency (35) which this subject revealed were partially corrected during corticotrophin ad-

TABLE I

Renal clearances

A. J.—surface area 1.50 m²

Date	Therapy	Inulin	PaH	F.F.
		cc./min.		
Mar. 20, 1951	control	55.4		
Jan. 2, 1952	control	53.4	298	0.18
Jan. 7, 1952	ACTH (day 4)	75.7	336	0.23
Jan. 12, 1952	control	40.5	261	0.15
Jan. 19, 1952	GH* (day 6)	56.3	268	0.21
Jan. 22, 1952	GH (day 9)	48.7	307	0.16
Jan. 26, 1952	GH (day 12)	51.0	293	0.17

Clearances are uncorrected for surface area.
* GH is growth hormone (Raben).

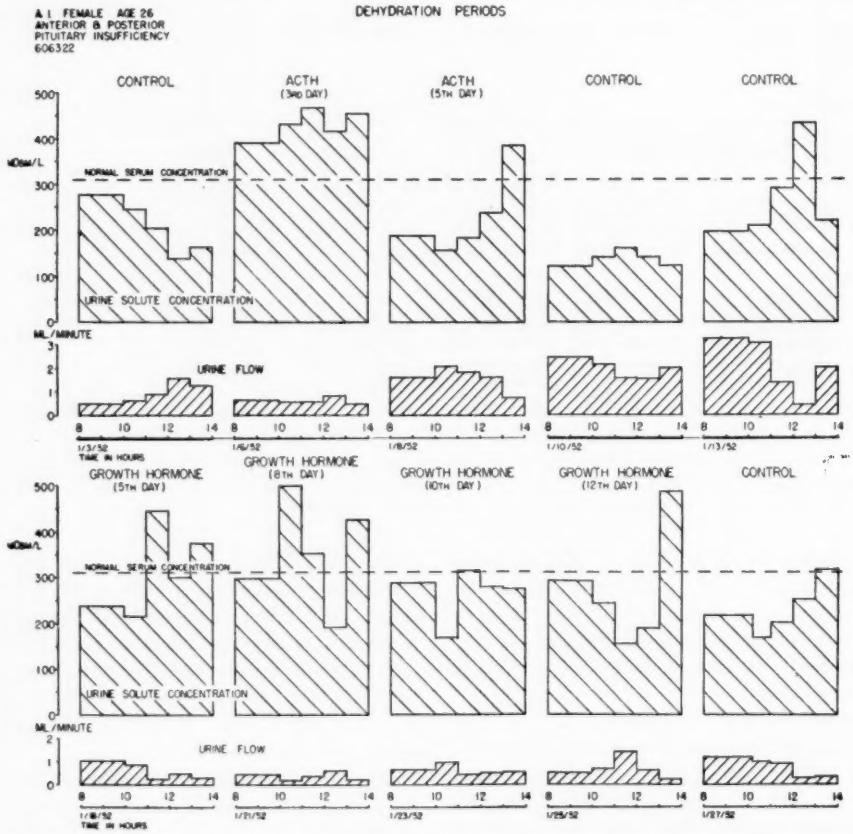

FIG. 5. THE RESULTS OF THE SAME STANDARD WATER DEPRIVATION AS DESCRIBED IN FIG. 4 SHOWING THE FAILURE OF ACTH AND GROWTH HORMONE TO LOWER URINE CONCENTRATION

The hypertonic urines on the third day of ACTH could have been due to slight contamination of the preparation with antidiuretic hormone. This possibility was excluded on the fifth day of ACTH by the duration of the test from the last dosages of ACTH although the eosinophile count at the end of the test was still only 4 per cmm. The sporadic occurrence of urines hypertonic to the serum remains unexplained.

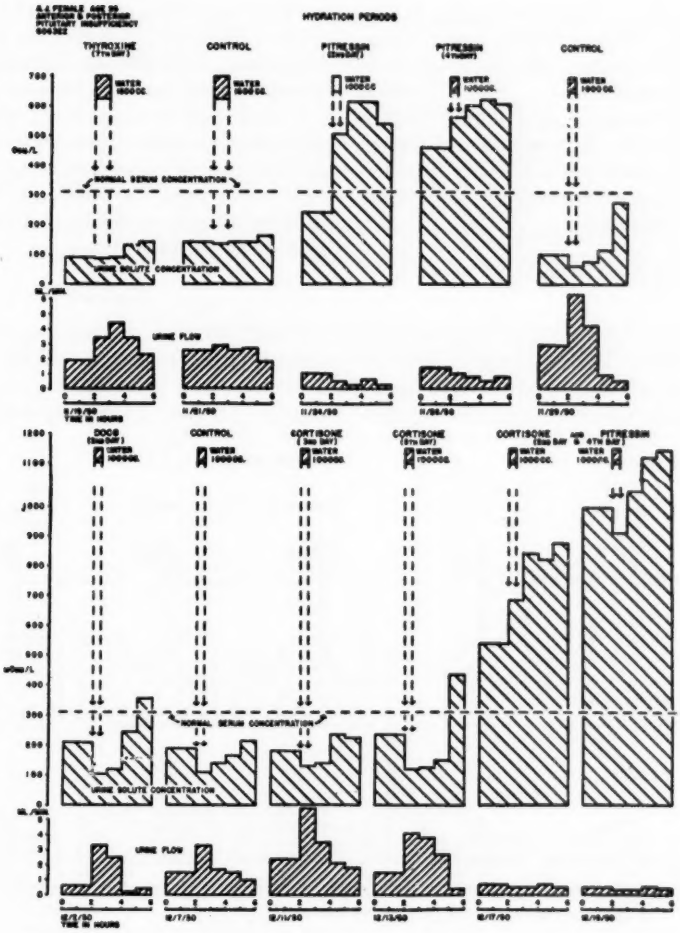

FIG. 6. THE EFFECTS OF THE VARIOUS ENDOCRINE PREPARATIONS UPON THE ABILITY TO HAVE A WATER DIURESIS

Note that with pitressin alone the water diuresis is abolished—urine concentration remains high and urine flow low after water ingestion. Water diuresis occurs during cortisone therapy but with cortisone and pitressin together diuresis is again abolished, and urines of maximal concentration are obtained.

ministration but unaffected by growth hormone (Table I).

To exclude the possibility that variations in fluid intake might be masking any diuretic effects of these hormones in the 24-hour urine collections, the patient was studied in each period under standard conditions of water deprivation. All fluids were withheld after 10:30 p.m. on the preceding night and urine collections were made from 6:30 to 8:30 a.m. and then at hourly intervals until 12:30 p.m. Serum was obtained for sodium and total solute concentration at 8:30 a.m. and 12:30 p.m. Breakfast without fluids was allowed. The urine concentrations in Figure 4 show no decrease during thyroxine, desoxycorticosterone or cortisone administration as compared with the control

periods. The apparent slight improvement in concentrating ability when cortisone was administered remains unexplained but this result is in the opposite direction from a diuretic effect. Figure 5 shows similar results during corticotrophin and growth hormone administration. Hence under the conditions of a nearly fixed excretory solute load no diuretic effect of any of these substances could be detected.

E. Effects of hormone administration on water diuresis

Figures 6 and 7 show the effect of administration orally of 1000 cc. of water on urine flow and urine concentration. It is evident that no significant enhancing effect on water excretion was produced by any of the administered hormones. In addition, Figure 6 shows that the antidiuretic effect resulting from pitressin administration was in no way reduced, but rather augmented when the same small dose of pitressin was administered together with cortisone. This observation suggests that the action of cortisone in correcting the defect of water excretion in Addison's disease is not mediated through an inhibition of antidiuretic hormone effect on the renal tubules.

DISCUSSION

Present methods for assessing insufficiency of anterior pituitary function are both indirect and insensitive. It is impossible to exclude completely anterior pituitary activity by current methods.

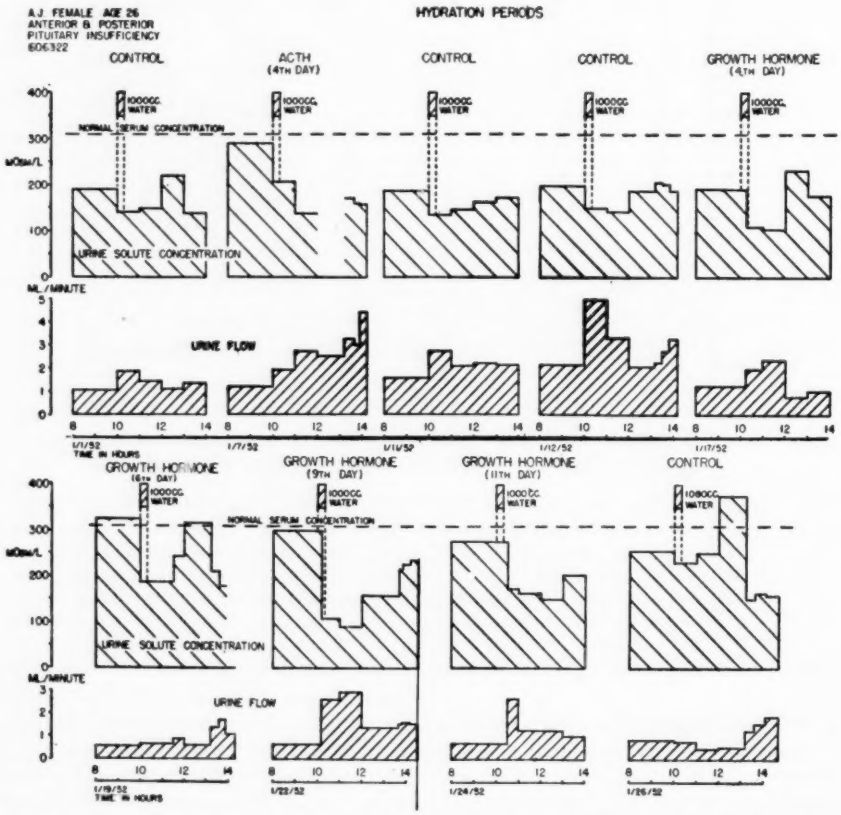

FIG. 7. SIMILAR TO FIG. 6 AND SHOWS NO SIGNIFICANT EFFECT OF ACTH OR GROWTH HORMONE IN ENHANCING A WATER DIURESIS

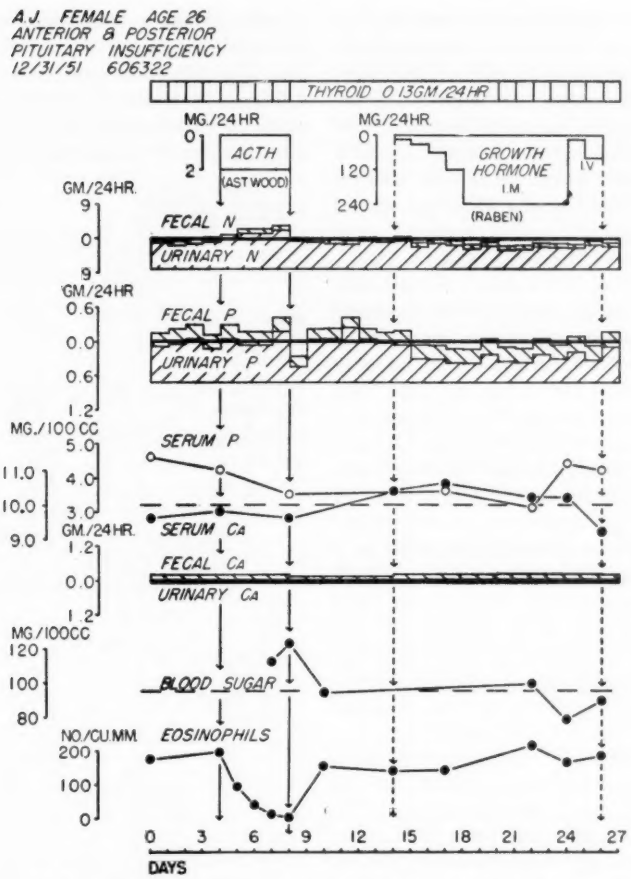

FIG. 8. METABOLIC DATA ON ACTH AND GROWTH HORMONE SHOWING NITROGEN AND PHOSPHORUS AND CALCIUM BALANCES, SERUM PHOSPHORUS AND CALCIUM, BLOOD SUGAR AND EOSINOPHILES

Note the retention of nitrogen and phosphorus (indicated by the clear area below the base line) that occurred during growth hormone administration (48). Growth hormone produced no hyperglycemic effect.

For purposes of this study it seemed sufficient that the anterior pituitary failure be associated with spontaneous disappearance of polyuria and polydipsia. In this regard the subject was comparable to the animals experimented on in which the diabetes insipidus state disappeared following destruction of various endocrine glands. The possibility remains, however, that only a slight residual anterior pituitary or adrenal cortical activity is essential to maintain the renal concentrating defect of neurohypophyseal failure and that the patient studied had this minimal residual function.

Winter and his associates (36) and Beaser (37) had previously pointed out that polyuria in diabetes insipidus is directly proportional to the excretory load. Richter (38) noted that the food intake of posthypophysectomized rats decreased concomitantly with their fluid exchange following destruction of their anterior pituitary gland. He surmised that diminution of water exchange might

simply represent the generalized metabolic depression that follows loss of anterior pituitary function. During the period of thyroxine administration, the subject complained of hunger. The increase in appetite which occurs during cortisone or adrenocorticotrophic hormone therapy is now a common clinical observation. It is likely that on a free diet our subject would have had an increased food intake and hence an increased solute excretion with this replacement therapy.

As recently stressed by Crawford, Schoen, and Nicosia (20), availability of solute may constitute a limiting factor in water diuresis. The maximal dilution of urine from normal human kidneys seems to be some 60 to 100 mOsm. per liter. Just as the normal human kidney cannot elaborate a urine during hydropenia above about 1200 mOsm. per liter, so also its ability to dilute the urine seems limited. Crawford and associates have shown that the decrease in rate of urine flow in the constantly water-loaded subject is probably the result of a shortage of solute available for excretion. Though the urine remains minimally concentrated, with a decreased rate of total solute excretion the rate of water excretion must also drop off. In our subject, whose urine was always near the lower limits of concentration, the urine volume was of necessity limited by the low rate of solute excretion.

White and Heinbecker have recently reported that growth hormone will re-establish the specific renal clearances in hypophysectomized dogs (39). deBodo and his coworkers reported only a moderate improvement of renal clearances in hypophysectomized dogs given growth hormone (40). In our subject growth hormone (Raben) failed to improve the depressed renal clearances (Table I). This lack of effect of growth hormone on renal clearances is to be contrasted with its anabolic effect in this subject—see phosphorous and nitrogen balances in Figure 8. This observation suggests that growth hormone effect is separable from any renotropic factor of the anterior pituitary.

When corticotrophin was administered there did occur an increase in clearances of about 50 per cent above the control levels in accordance with the findings of Burnett (41). With urines of minimal concentration such as this subject excreted an increase of water reaching the distal tubules as a result of augmented filtration rates could only be excreted if a proportionately greater amount of solute were also available for excretion. A persistent excretion of solute in excess of intake would very soon result in marked deficits of body solute contents. Theoretically it seems inescapable that so long as urines are already minimally concentrated no factors can in-

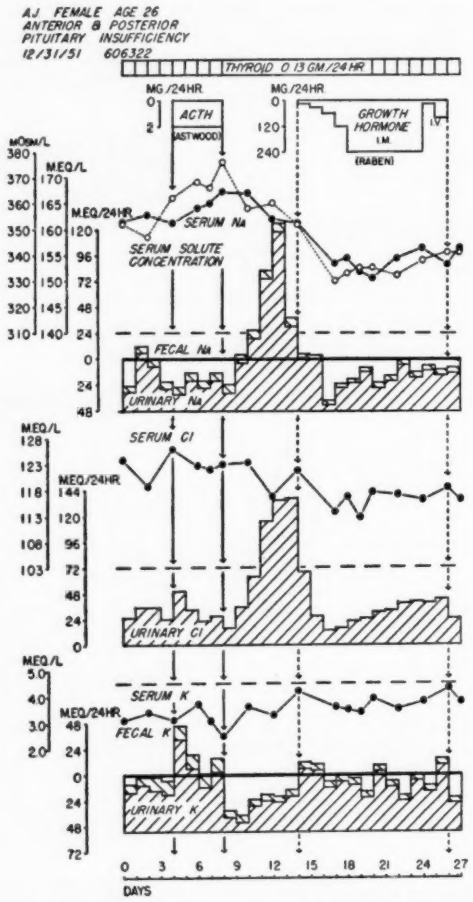

FIG. 9. METABOLIC DATA ON ACTH AND GROWTH HORMONE SHOWING SODIUM AND POTASSIUM BALANCES, URINARY CHLORIDE EXCRETION, AND SERUM TOTAL SOLUTE, SODIUM, CHLORIDE AND POTASSIUM CONCENTRATIONS

Note the abnormally high serum sodium, total solute and chloride concentrations throughout the study (see text). The serum potassium was abnormally low. The control period between ACTH and growth hormone was too brief to permit any interpretation of the sodium and chloride changes that occurred during growth hormone administration.

crease diuresis which do not, over a prolonged period of time, cause an increase in the intake of solutes which will eventually be excreted through the kidneys. Handley and Keller have appropriately suggested that the decrease in renal clearances subsequent to hypophysectomy serves as a defensive mechanism preventing depletion of body constituents (42).

It was somewhat of a surprise to find the subject excreting only minute amounts of sodium in the urine during the initial control period and following the pitressin period in spite of a daily sodium intake of 50 mEq. and an elevated serum sodium concentration. Such a degree of renal conservation of sodium probably indicates some residual adrenal cortical activity (43) and has been reported in totally hypophysectomized dogs (44). This pattern of a high serum sodium concentration with only small amounts of sodium in the urine is similar to that of the "dehydration reaction" characteristic of simple water restriction (45, 46). During the solute loading period, serum sodium, chloride, and total solute concentrations rose to alarming levels and drowsiness, apathy and anorexia on the fourth day caused refusal of part of the diet and sodium chloride-urea mixture. The failure of the patient to ingest sufficient water while on an *ad libitum* water intake to correct this hypertonicity of her serum suggests that her "thirst center," as well as her pituitary, was damaged.

That the hormones tested had no direct renal effect on water excretion in this subject with neurohypophyseal failure, obviously does not exclude the possibility that with some residual neurohypophyseal function these hormones might be effective through inhibiting the release of antidiuretic hormone or interfering with the action of antidiuretic hormone on the renal tubule. The latter possibility has been excluded in the present study only in the case of cortisone. That the subject with Addison's disease during treatment with cortisone can elaborate urines of maximal concentration during water deprivation (47) indicates that cortisone does not interfere with the normal release of antidiuretic hormone.

SUMMARY AND CONCLUSIONS

1. A patient with diabetes insipidus due to known neurohypophyseal insufficiency who subsequently developed evidence of some anterior pituitary failure is presented.

2. Spontaneous disappearance of polyuria and polydipsia occurred concomitantly with dysfunction of the anterior pituitary thus simulating the situation in the animal with experimental diabetes insipidus whose water exchange returns toward normal following ablation of the anterior pituitary.

3. In spite of the disappearance of polyuria the physiological defect which characterizes neurohypophyseal insufficiency persisted. This defect is an inability to elaborate a concentrated urine in response to the stimulus of an elevated effective total solute concentration of the serum.

4. The disappearance of polyuria could be satisfactorily explained in this case as resulting from a decrease in the excretory solute load. When the excretory solute load was increased, polyuria recurred.

5. When the excretory solute load was kept nearly fixed by adherence to a constant diet, the administration of thyroxine, desoxycorticosterone glucoside, cortisone, corticotrophin, and growth hormone could not be shown to have any effect on water excretion. The urine concentration did not fall and volume did not increase.

6. It is suggested that any diuretic effects previously noted for these substances were secondary to their general effects of increasing metabolism, activity, food intake, and hence the excretory solute load. The possibility that they may interfere with the release or action of antidiuretic hormone also exists.

7. Cortisone administration in no way diminished the antidiuretic effect of pitressin injections.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Drs. James H. Means and Walter Bauer whose interest and support made possible this study. Their thanks go also to Dr. Fuller Albright, whose patient was the subject for this study, and to Dr. J. D. Crawford, who generously made available his freezing point depression apparatus.

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ABSORPTION OF GLUCOSE AND METHIONINE FROM THE HUMAN INTESTINE; THE INFLUENCE OF THE GLUCOSE CONCENTRATION IN THE BLOOD AND IN THE INTESTINAL LUMEN

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(Submitted for publication June 11, 1952; accepted August 1, 1952)

INTRODUCTION

The inaccessibility of the absorbing surface of the small bowel and its blood supply has made studies of intestinal absorption difficult and has left large gaps in our knowledge of the fundamental mechanisms involved and the factors influencing them. Animal experimentation has provided the basis for much of what is known (1). The desirability of obtaining similar data in the human subject has led to many ingenious methods for the measurement of absorption in man. None of these, however, has proved entirely satisfactory. Studies of systemic blood concentrations of ingested materials reflect utilization, rate of storage, excretion, and circulatory competency as well as absorption. Recent methods using the sampling of "portal" blood from anastomotic abdominal vessels in cases of portal hypertension employ as subjects patients with cirrhotic livers, in which the effects of the portal hypertension on absorptive processes, and the extent and effect of dilution of intestinal venous blood with hepatic arterial blood are unknown (2–4). Balance studies require elaborate supervision, are expensive, and are inapplicable to the study of absorption of sugars, due to destruction from fermentation.

Various techniques using intubation of the human small bowel have provided a more direct approach to the subject (5–7). Disturbance of normal intestinal functioning has led to objections to several of these procedures (8). Nicholson and Chornock (9) seem to have devised the most physiologic of the intubation methods for the measurement of absorption. In their technique the material to be studied is dripped into the intestine, travels downstream with the normal bowel contents, and is aspirated below, loss beyond the point of aspiration being prevented by an occluding balloon. The advantages are normal admixture with intestinal contents, maintenance of normal ratio of volume of material to mucosal area, physiologic entrance of material into the bowel, lack of interference with normal peristalsis in the absorbing area, and a fairly sharp starting and stopping point. These authors reported the application of their method to the study of ascorbic acid absorption.

It has been our purpose in this investigation to study some additional phenomena of human intestinal absorption by the Nicholson-Chornock method, and to evaluate its use as an investigative tool. In this paper is presented our experience in studying the absorption of two different concentrations of glucose and methionine solutions from the upper small bowel of normal human subjects, and the effect of insulin-induced hypoglycemia and of hyperglycemia on the absorption rate.

METHODS

In place of a two-lumen tube with an additional small outer tube to control inflation of the balloon, as employed by Nicholson and Chornock, a single, three-lumen, size 16 French tube was used. The first lumen connected with the balloon, which was located on the end of the tube. The second lumen communicated with six aspiration holes, immediately proximal to the balloon. Forty-five centimeters proximal to these were located two additional holes which connected with the third lumen.

All subjects were intubated in the morning after an 8 hour fast. When the two most proximal (inlet) holes were in the midduodenum (as judged by fluoroscopy and the aspiration of bile-stained alkaline fluid) the tube was anchored to the nose and the test begun. The distal end of the tube with the balloon was now well into the upper jejunum.

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The subject was placed in the supine position and the balloon was inflated to a pressure of 25–30 centimeters of water as measured on a manometer which was provided with a float so that intestinal motility patterns could be kymographically recorded during the test. The solution to be used in the experiment was then allowed to drip into the proximal (inlet) holes in the duodenum at a rate of 10 cc. per minute, which simulates the normal entrance rate of material into the bowel from the stomach as determined by Karr and Abbott (10). The rate of infusion was controlled by a "Flowrator."[2] As the infusion was started, suction was applied to the outlet holes 45 cms. down the bowel and maintained by a pump at a negative pressure of 125 cm. of water. Timings were made by stopwatch. After 30 minutes the infusion was stopped and the bottles changed on the suction lumen. Three 10 minute "wash" periods were then run, with an injection of 100 cc. of water or 33 per cent Mg SO_4 through the inlet holes at the beginning of each period, the aspirated samples being collected separately. This constituted one "absorption test." In 20 to 30 minutes a second complete test was carried out and, if any changes in the experimental conditions were desired (injection of insulin or intravenous glucose, for instance) they were introduced just prior to the start of the second test. It was thus possible to vary the conditions using the subject as his own control. At the end of the entire procedure the position of the tube was again verified fluoroscopically before removal.

In some tests it was desired to check on possible loss of the infusing solution past the occluding balloon. A four-lumen, size 18 F tube was used, constructed as above, with the fourth lumen connected to holes located distal to the balloon. In such tests constant suction was maintained on these openings during the entire procedure.

The substances used in the absorption tests were glucose and *dl*-methionine in solutions containing approximately either *1*) 5 per cent glucose and 0.25 per cent methionine or *2*) 10 per cent glucose and 0.50 per cent methionine. Approximate concentrations only were used because the magnitude of other errors inherent in the procedure did not seem to justify further refinement. In each case, however, the solutions were analyzed to determine their exact composition. Preliminary tests revealed no interference in the chemical determination of either of these substances by the other in the concentration ratios used.

When the tests were completed, all samples including the infusion solution were measured and analyzed for glucose by the colorimetric Folin-Wu method, and for methionine by the Hess and Sullivan modification (11) of the original McCarthy and Sullivan method. In certain cases the specimens during the methionine analysis gave turbid solutions, and these cases were discarded as yielding inaccurate results. In only a few cases a "blank" reading for methionine was given by a specimen of pure intestinal juice obtained prior to beginning the test. This "blank" was then subtracted from the amounts contained in the specimens. In no case was there found a "blank" reading for glucose. When blood was analyzed for glucose, the micro method of Nelson (12) was used which employs capillary blood. The Klett photoelectric colorimeter was used for all determinations.

In calculating the results of the experiments the total amounts of material aspirated during the initial 30-minute infusion period and during the three 10-minute wash periods were substracted from the amount introduced in the first 30-minute period. The result is expressed as amount of material absorbed per 30 minutes.

The subjects studied were either healthy young male medical students, or patients from the wards of the New York Hospital without known gastrointestinal disease or alterations in metabolism. All had been taking a balanced diet in the days immediately preceding the tests and reported to the laboratory in the fasting state.

OBSERVATIONS

1. *Relation of intestinal motility to the sharpness of the end-point of the procedure*

Typical complete results for two representative tests are demonstrated in Table I. Only the figures for glucose are given for the sake of simplicity. In the first subject, T. M., 5 per cent glucose was studied and it will be seen that the washing procedures were quite effective in that all of the glucose was removed with the first wash, providing a fairly sharp "end-point" to the procedure. In the second subject, L. E., however, in which 10 per cent glucose was used, it will be seen that some glucose was still washed out with the final rinse. When calculating the amount absorbed per 30 minutes, there is the obvious error of glucose having been present on the absorbing mucosa for considerably longer, though in decreasing concentrations. Fortunately, over 75 per cent of our tests corresponded to the first type with fairly sharp end-points.

The reason for this delay in evacuating all the non-absorbed material in some of the tests does not seem to be due to the somewhat smaller bore suction tube used in place of the original Nicholson-Chornock model because, while their tube had a withdrawal capacity of 175 cc. per minute, ours had a capacity of 100 cc. per minute, well in excess of the possible rate of accumulation. Nor does it seem related to the use of water as a wash material in place of the recommended magnesium sulfate, which the original authors believed to cause

[2] Flowrator (Graubard-Peterson) manufactured by Fischer & Porter Company, 350 Fifth Avenue, New York.

TABLE I

Complete results of two representative tests of glucose absorption

Subject	Infusion 1) Gluc. conc. 2) Vol. 3) Total gluc.	Fluid vol. aspirated	Gluc. conc. in aspirated samples	Amount gluc. aspirated	Total gluc. absorbed
T. M.	1) 5.30 gm. % 2) 315 cc. 3) 16.70 gm.	1) 30 min. = 325 cc. 2) 1st wash = 113 cc. 3) 2nd wash = 106 cc. 4) 3rd wash = 85 cc.	1) 2.46 gm. % 2) 1.46 gm. % 3) .04 gm. % 4) .00 gm. %	1) 8.00 gm. 2) 1.65 gm. 3) — 4) — Total 9.65 gm.	7.05 gm.
L. E.	1) 10.55 gm. % 2) 296 cc. 3) 31.20 gm.	1) 30 min. = 140 cc. 2) 1st wash = 93 cc. 3) 2nd wash = 77 cc. 4) 3rd wash = 66 cc.	1) 3.32 gm. % 2) 3.72 gm. % 3) 1.74 gm. % 4) .30 gm. %	1) 4.65 gm. 2) 3.46 gm. 3) 1.34 gm. 4) .20 gm. Total 9.65 gm.	21.55 gm.

a peristaltic rush down the bowel thus providing a rapid evacuation of unabsorbed material. With the method of simultaneously recording motility no evidence of increased peristalsis was found in those tests employing magnesium sulfate, and washing with water seemed to be just as satisfactory. Rather the differences in efficiency of washing are more closely related to differences in the spontaneous bowel motility at the time. Figure 1 shows the motility tracing obtained in the two tests on T. M. and L. E. Whereas that of T. M. was quite active, the motility of the bowel in L. E. was relatively sluggish. Evidently intestinal peristaltic activity is needed to "milk" the washing fluid down to the evacuation openings. It was possible to predict that one would obtain a "good" test or a "bad" one by watching the degree of motility being recorded.

2. *Possible loss of material past the occluding balloon*

In all experiments, a condom rubber balloon approximately 2 inches long, inflated to a pressure of 25 to 30 cm. of water, was used. Abbott and Miller (6) have shown that under such conditions the small bowel lumen is effectively blocked. To check this further, four subjects were studied at the end of their absorption tests. Thin suspensions of barium were injected through the holes just proximal to the balloon and fluoroscopic observations were made for 30 minutes afterward. In no case was the barium seen to leak past the balloon. As an additional check, the four-lumen tube was used in eight subjects for a total of 16 absorption tests. Though suction was maintained during the entire procedure (in some of which intestinal motility was stimulated with drugs),

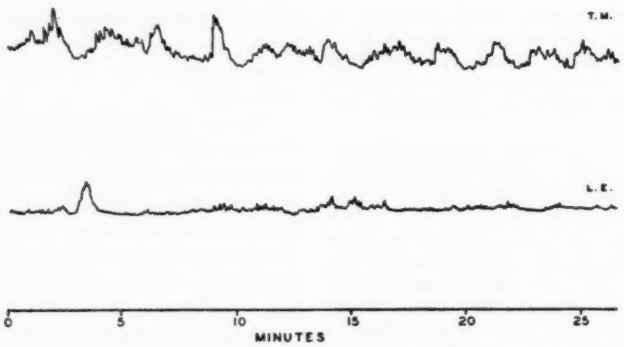

FIG. 1. MOTILITY TRACINGS FROM THE SMALL INTESTINE, INDICATING DIFFERENCES IN SPONTANEOUS PHASIC ACTIVITY AMONG VARIOUS TESTS

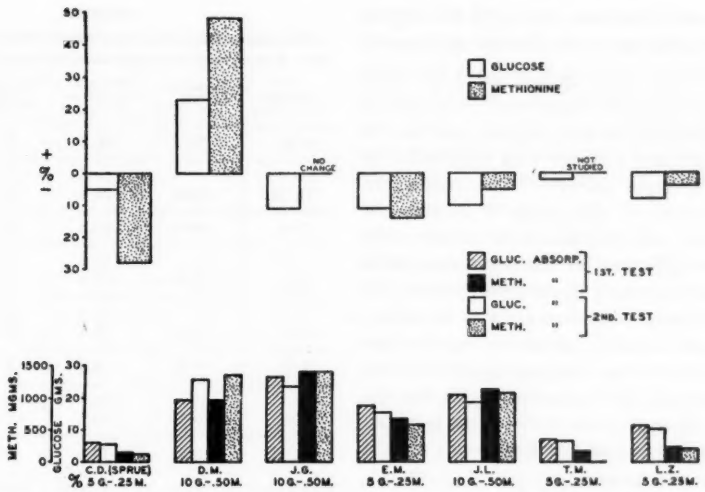

FIG. 2. ABSOLUTE ABSORPTION RATES IN SEVEN SUBJECTS AS DETERMINED BY TWO CONSECUTIVELY PERFORMED TESTS WITHOUT MEDICATION

Percentage of change between the two tests is indicated above.

no glucose or methionine was found in any of the fluids collected distal to the balloon. In addition, we had the opportunity of studying ileal absorption in one patient with an ileostomy. In this subject the distal end of the tube was located just proximal to the ileostomy, and throughout the absorption tests the ileostomy bag was kept in position. The contents of the bag remained free of glucose and methionine. Evidently the balloon provides an adequate block preventing loss of material past it.

3. *Difficulty in maintaining tube position in the bowel*

Fluoroscopic visualization of the tube before beginning the absorption test and again at the end of the procedure, demonstrated that, in a fair proportion of the cases, there was a tendency for the intestine to move up along the tube. The second observation often showed the tube tip down nearer the cecum, although that portion of it remaining outside the subject stayed the same, and the tube was firmly anchored. Evidently in some of the cases, the 45 cm. length of intestine whose absorption rate was being studied did not remain always the same.

4. *Variability in the results of two absorption tests performed consecutively*

All tests on all subjects were run consecutively in duplicate. In seven subjects no attempt was made to alter conditions between the two tests in order to determine the reproducibility of results. The glucose and methionine absorption rates in these subjects are shown in Figure 2. The subject C. D. was a patient with sprue and his absorption figures will not be considered in the remainder of this paper. The greatest variation between two tests for glucose absorption is under 25 per cent and for methionine absorption, under 50 per cent. Most of the tests show considerably less variability. Thus, by studying the absorption rate in the same individual, and under the same circumstances, and within a short period after a previous study, the checks are gratifyingly close, considering the many potential sources of error. Six of the seven subjects show an absorption rate in the second test below that in the first. That this might be due to the fact that the subject approached the second test with blood levels of glucose and methionine higher than he had had in the preceding test, seems unlikely in view of subsequent studies of the effect of hyperglycemia.

5. *Difference in absorption rates with two different concentrations of glucose and methionine*

Absorption rates were studied for 5 per cent glucose and 0.25 per cent methionine in 16 and 15 instances respectively in nine subjects, and for 10 per cent glucose and 0.50 per cent methionine in 12 instances in eight subjects. The results are shown in Tables II–V. The range of amounts absorbed of both test substances is considerable. However, the difference in the mean absorption values for 5 per cent and 10 per cent glucose (6.2 gm.) is 3.26 times the standard error of the difference, and the difference in means for the two concentrations of methionine (586 mgm.) is 4.92 times its standard error. For a sample of this size the difference in absorption rate between the two concentrations of both substances thus seems to be a real one and substantiates the view that the intestinal mucosa can absorb glucose and methionine at a greater rate from 10 per cent and 0.50 per cent solutions than from 5 per cent and 0.25 per cent concentrations respectively.

TABLE II

Absorption rates for approximately 5 per cent glucose

Subject	Conc. test sol. gm. %	Gm. gluc. absorbed 30 min.	% Gluc. absorbed	Gluc. absorption coefficient*
E. M.	6.14	17.7 15.7	98 93	8.5 7.5
H. R.	6.38	13.4	68	6.6
T. M.	5.30	7.1 6.9	42 45	3.6 3.6
P. S.	6.00	17.9	88	9.4
A. B.	5.70	13.1 14.9	73 87	8.8 9.9
R. Q.	5.46	17.7 17.0	86 96	8.8 8.4
H. V.	5.46	10.1 10.2	59 60	5.7 5.7
T. M.	5.78	12.5 10.6	74 64	6.4 5.4
L. Z.	5.46	11.3 10.4	69 64	6.8 6.2
Range		6.9–17.9	42–98	3.6–9.9
Mean ($\bar{X}$)		12.9	73	7.0
Stan. dev. (σ)		±3.6		±1.9
Coeff. of var. $\left(\frac{100\,\sigma}{\bar{x}}\right)$		28%		27%
Stan. error mean ($\sigma_{\bar{x}}$)		±.90		±.48

* Absorption coefficient = gm. absorbed/30 min./sq. meter body surface.

TABLE III

Absorption rates for approximately 10 per cent glucose

Subject	Conc. test sol. gm. %	Gm. gluc. absorbed 30 min.	% Gluc. absorbed	Gluc. absorption coefficient
P. S.	9.75	14.2	52	7.5
D. M.	10.45	19.6 25.9	70 88	9.7 12.8
C. T.	10.00	10.5	39	5.9
J. G.	10.45	26.6 23.8	94 75	13.3 11.9
J. L.	10.40	21.1 18.9	64 59	11.2 10.0
S. B.	9.25	7.3	27	4.1
L. E.	10.55	21.6 20.9	69 73	14.1 13.6
K. M.	10.55	19.3	60	8.8
Range		7.3–26.6	27–94	4.1–14.1
Mean ($\bar{X}$)		19.1	64	10.2
Stan. dev. (σ)		±5.8		±3.2
Coeff. of var. $\left(\frac{100\,\sigma}{\bar{x}}\right)$		30%		31%
Stan. error mean ($\sigma_{\bar{x}}$)		±1.67		±.92

In calculating "absorption coefficients" an attempt was made to reduce the scatter of the results by reducing all values to amounts absorbed per 30 minutes per square meter of body surface. The results seem to indicate, however, that no reduction in variability is achieved. The variability of the "absorption coefficients" is quite similar to that of the uncorrected figures. Apparently variation in body surface area is not one of the factors contributing to the scatter of the absorption rates.

In the range of concentrations studied, the percentages of glucose and methionine absorbed are roughly the same. That this correlation is not due to loss or lack of complete recovery of unabsorbed material, is demonstrated by the evidence that loss past the balloon does not occur and by the fact that, in all cases, the three wash periods effectively removed all unabsorbed residue, though

TABLE IV

Absorption rates for approximately 0.25 per cent methionine

Subject	Conc. test sol. gm. %	Mgm. meth. absorbed 30 min.	% meth. absorbed	Meth. absorption coefficient*
E. M.	.26	684 589	89 82	326 281
H. R.	.30	512	55	251
T. M.	.24	186	24	96
P. S.	.24	476	59	250
A. B.	.26	583 578	71 74	389 386
R. Q.	.24	696 728	77 93	345 361
H. V.	.20	298 323	48 52	167 180
T. M.	.26	307 396	41 53	157 203
L. Z.	.20	231 222	39 38	138 133
Range		186–728	24–93	96–389
Mean ($\bar{X}$)		454	60	244
Stan. dev. (σ)		±172		±99
Coeff. of var. $\left(\frac{100\,\sigma}{\bar{x}}\right)$		38%		41%
Stan. error mean ($\sigma_{\bar{x}}$)		±44.4		±25.6

* Absorption coefficient = mgm. absorbed/30 min./sq. meter body surface.

the speed of this recovery varied, as demonstrated above.

6. *Effect of hyperglycemia and of insulin-induced hypoglycemia on the absorption of glucose and methionine*

The effect of hyperglycemia on the absorption rates was studied in three subjects. The procedure was to perform one control test, and after an interval of 20 to 30 minutes, an intravenous injection of 50 cc. of 50 per cent glucose was given and, immediately afterward, the second absorption test was begun. Capillary blood sugar determinations were made at the beginning and end of each 30-minute test. In one subject (A. B.) 5 per cent glucose and 0.25 per cent methionine was used and in another (M. M.) 5 per cent glucose was used alone. In the third subject (L. E.) 10 per cent glucose and 0.50 per cent methionine was studied. Two of the subjects had the tube located in the usual region of the upper small bowel. The third subject (M. M.) had an ileostomy and the tube was lowered into the ileum so that the distal balloon was just proximal to the ileostomy. Because values for absorption rate in this case represent ileal absorptive capacity, they are not included in the tables. The results obtained in these three experiments are shown in Figure 3. Despite the fact that the average blood sugar level during the second test is considerably higher than during the first, the percentage of change in absorption of glucose and methionine is well within the range of normal variation between two tests as illustrated in Figure 2.

In three additional subjects the influence of insulin-induced hypoglycemia on the absorption rates was studied. The procedure here was to perform one control test and, after an interval of 20 to 30 minutes, the subject was given an intravenous injection of insulin. Fifteen minutes later, the

TABLE V

Absorption rates for approximately 0.50 per cent methionine

Subject	Conc. test sol. gm. %	Mgm. meth. absorbed 30 min.	% meth. absorbed	Meth. absorption coefficient
P. S.	.60	912	54	480
D. M.	.65	983 1451	56 79	485 715
C. T.	.45	470	39	264
J. G.	.55	1398 1400	94 83	700 700
J. L.	.50	1148 1096	72 71	605 575
S. B.	.60	486	28	270
L. E.	.50	1237 994	84 74	805 650
K. M.	.50	900	59	409
Range		470–1451	28–94	264–805
Mean ($\bar{X}$)		1040	66	555
Stan. dev. (σ)		±384		±176
Coeff. of var. $\left(\frac{100\,\sigma}{\bar{x}}\right)$		37%		32%
Stan. error mean ($\sigma_{\bar{x}}$)		±110.7		±50.8

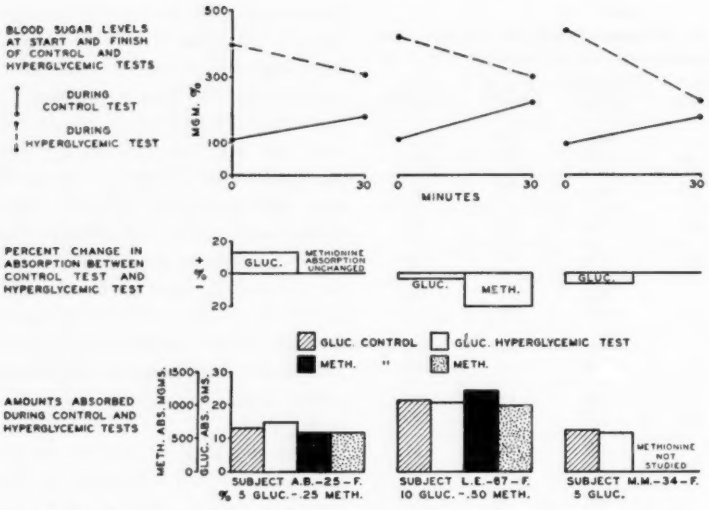

FIG. 3. EFFECT OF HYPERGLYCEMIA ON ABSORPTION RATES OF GLUCOSE AND METHIONINE

second absorption test was begun. Blood sugar determinations were made in both tests as indicated in Figure 4. In all cases 5 per cent glucose and 0.25 per cent methionine was used. One subject (R. Q.) was given 20 units of regular insulin, the other two subjects were given 25 units. It is apparent that, although a considerable fall in blood sugar occurred during the second tests, the changes in glucose and methionine absorption rates are again within the normal range of variation.

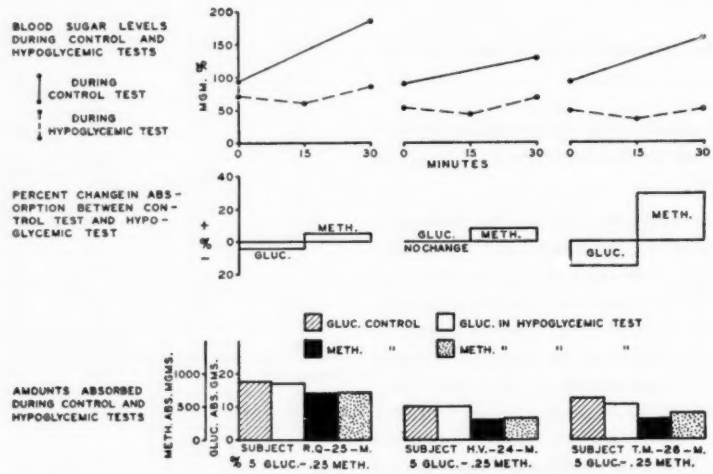

FIG. 4. EFFECT OF HYPOGLYCEMIA ON ABSORPTION RATES OF GLUCOSE AND METHIONINE

DISCUSSION

Experience with the study of human small bowel absorption using the Nicholson-Chornock principle demonstrates that it is a practical procedure which can be used in investigations of certain aspects of absorption, but that it has definite technical and physiological limitations. From the standpoint of the technique itself, the procedure leaves several variables, which can be only partially controlled. Movement of the tube along the bowel (or the bowel along the tube), seems to be an uncontrollable variable so that the segment of bowel being studied is not necessarily always the same. Since the comparative absorption rates of various levels of the small bowel in man have not been adequately studied, the effect of this factor is unknown. Although we have not directly observed this, it also seems highly probable that, at times, the bowel may "telescope" along the tube, thus presenting an absorbing surface greater than the 45 cm. interval between the tube fenestrations would indicate. The difficulties in obtaining a sharp end-point to the procedure naturally tend to destroy the accuracy of assigning a given absorption value to a set period of time. This lack of sharp end-point has been quite closely correlated with sluggish bowel motility.

Despite these technical objections, the procedure does seem to duplicate physiologic conditions more closely than other previous direct methods of absorption study, and, in our experiments, the reproducibility of results in two consecutive tests has been gratifying. The variation between two tests for glucose has been about 25 per cent, and for methionine about 50 per cent; yet the range in absorption rates from subject to subject is much higher. We are thus forced to the conclusion that the main factor in this marked variability is not the fault of the technique itself, but is rather due to actual absorptive differences among individuals (13). From the practical standpoint of the investigator these results would appear to indicate that the method is of chief use in studying those aspects of absorption which can be varied between two consecutive tests.

Cori's original studies of glucose absorption in rats (14) showed that the absorption rate was constant for solutions of from 25 per cent to 80 per cent. This work formed the basis for the so-called "Cori's law" to the effect that the absorption rate of sugars is independent of their concentration. Hewitt (15), also studying rats, has shown that for very dilute solutions (below 5 per cent) the rate varies directly with the concentration. Studying this problem in the human being, Abbott, Karr, and Miller (5) have demonstrated a similar phenomenon for glucose concentrations of below 5 per cent and Groen (7) has shown, in a few cases, that there is a direct relationship of concentration to absorption rate up to about 10 per cent, and that over this concentration the rate remains about the same. He did not study a sufficient number of cases, however, to do more than suggest this relationship. Our experiments with two different concentrations of glucose and methionine have been of sufficient number to show a statistically significant greater rate of absorption for the more concentrated solutions. It is apparent, then, that for the range of concentration of glucose naturally occurring in the human small bowel (5), "Cori's Law" does not apply, and that absorption of glucose can be enhanced by increasing its concentration from 5 to 10 per cent. This same phenomenon applies to the representative amino acid methionine in the solution strengths studied. In this connection, it is interesting to speculate on a possible contributing factor in the poor absorption in sprue. The hypomotility of the bowel in sprue, including the stomach in some cases, is well known (16, 17). Warren and his co-workers (18) have shown that the stomach dilutes hypertonic sugar solutions before releasing them into the duodenum. It seems plausible to assume, therefore, that delayed gastric emptying in sprue may sufficiently dilute ingested foodstuffs to present to the small bowel a concentration below optimum for maximal absorption. Perhaps lending credence to this speculation is the observation by May and McCreary (17) that the glucose tolerance curve in children with celiac disease could sometimes be improved by delivering the glucose directly into the small bowel by duodenal tube. That the intestine itself, however, exhibits a deficient absorption rate in sprue has been shown in one subject by Groen (19) and in two subjects in the course of our studies.

No previous direct observations on the absorption of methionine from the human small bowel

seem to have been carried out. Althausen and his associates (20, 21) have employed methionine tolerance tests in studying the effects of massive bowel resection on human intestinal absorptive capacity, and Chase and Lewis (22) and Hess (23) have studied directly methionine absorption from the bowel of rats. In none of these studies has the effect of varying concentrations on absorption rate of this amino acid been observed.

In our six experiments wherein the effect of variation in blood sugar was studied, no direct influence of hyperglycemia or hypoglycemia on absorption could be demonstrated. Since the magnitude of the change would have to be more than 25 per cent for glucose or 50 per cent for methionine for its appreciation, it is possible that small changes in absorption may have occurred which are within the error of the method. In this connection, Cori (24) has demonstrated in rats that insulin administration increased the absorption rate for glucose by only about 6 per cent. Were the processes of absorption concerned only with diffusion, it might be expected that the relatively minor changes in blood glucose levels produced here would scarcely alter the gradient between the relatively enormous concentration in the bowel lumen and that in the blood perfusing the mucosa. Since we know that glucose, at least, is absorbed by an active process, it does not seem unreasonable to expect a change in absorption rate depending on body need as reflected by blood concentrations. Our results indicate, however, that the intestinal mucosa does not alter significantly its absorption of glucose with depression or elevation in the blood sugar level. Likewise, Nicholson and Chornock (9) found no difference in the absorptive rate for ascorbic acid between individuals with low and high plasma values for this vitamin, and Mosely and Chornock (25) failed to demonstrate any reduction in galactose absorption following the previous administration of glucose.

SUMMARY AND CONCLUSIONS

1. An evaluation of the Nicholson-Chornock procedure for the study of human small bowel absorption is reported, based on its use in eighteen subjects for the study of glucose and methionine absorption. The technique appears to be of definite value in the investigation of certain aspects of absorption, but has several sources of error which limit its usefulness.

2. The variability in results between two consecutively run tests in seven subjects was under 25 per cent for glucose and 50 per cent for methionine. The variability in results from individual to individual is several times as large, suggesting that the greatest contributing factor in such a range lies in variation in the absorptive capacity of the intestine itself and not in technical difficulties involved in performing the test.

3. The chief investigative use of the procedure, therefore, seems to lie in the study of those influences on absorption which can be varied between two consecutively performed tests, rather than in comparing absorption rates in one control group with those in another experimental group.

4. The mean rate of glucose absorption by a 45 cm. segment of upper small bowel in man for a 30-minute period, under the conditions of our procedure, is 12.9 gm. from a 5 per cent solution, and 19.1 gm. from a 10 per cent solution. Similar rates for *dl*-methionine are 454 mgm. for a 0.25 per cent solution, and 1,040 mgm. for a 0.50 per cent solution. These differences are both statistically significant and disprove the application of "Cori's law" to the absorption of glucose in the range of concentrations naturally occurring in the human intestine.

5. Within the limits of sensitivity of the procedure no detectable influence on the absorption rate of glucose or methionine could be demonstrated by elevating the blood sugar with intravenous glucose or reducing it with insulin.

ACKNOWLEDGMENT

The author wishes to express his gratitude to Dr. Thomas P. Almy for his many valuable suggestions and constant encouragement, and to Miss Louise Wallace for technical assistance.

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MEASUREMENT OF GLOMERULAR FILTRATION RATE IN CHILDREN WITH KIDNEY DISEASE [1, 2]

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(Submitted for publication July 7, 1952; accepted August 1, 1952)

The renal clearance of inulin is generally accepted as a measure of glomerular filtration rate (GFR) of water in normal subjects (1). If specific methods (2, 3) are used to determine endogenous creatinine in serum and urine, the creatinine: inulin clearance ratio is close to unity in normal infants, children (4) and adults (5). However, in patients with diminished kidney function, the creatinine [4] clearance is consistently higher than the clearance of inulin (4–7). This fact suggests that in patients with kidney disease, there may be either tubular secretion of creatinine, tubular reabsorption of inulin, or both. The present observations were designed to investigate more completely than had been done previously (8) the problem of whether the clearance of inulin or of creatinine provides a valid measure of glomerular filtration rate in children with kidney disease.

SUBJECTS AND METHODS

Clearances of inulin (C_{IN}) and creatinine (C_{CR}) were measured simultaneously in 57 children with kidney disease (40 patients with the nephrotic syndrome, 15 patients with glomerulonephritis, and two patients with congenital renal anomalies) whose ages ranged from 1½ to 15½ years and in 11 children without kidney disease whose ages ranged from 4 to 12½ years. In some of the observations, clearances of one or more of the following substances were also measured: *p*-aminohippurate (C_{PAH}),[5] urea (C_U), thiosulfate (C_{THIO}), and glucose (C_G). Clearances were measured before and after administration of Carinamide [5] (4'-carboxyphenylmethanesulfonanilide) in four children with kidney disease; before and after injection of desoxycorticosterone glucoside (DCG) [5] in two children with and one without kidney disease; at both low and high concentrations of serum PAH in five children with kidney disease; and at three different concentrations of serum inulin in two children with and one without kidney disease. In the latter observations, step-wise increases in serum inulin concentrations were achieved by increasing the concentration of inulin in the infusion fluid which was administered intravenously at a constant rate. The catheterization and infusion technique used for short-term simultaneous clearances has been described previously (6, 9). Inulin was measured in serum and urine using resorcinol as described by Schreiner (10); PAH by the method of Smith and his associates (11); thiosulfate as described by Newman, Gilman and Philips (12); urea by the method of Archibald (13); and glucose as described by Nelson (14). Carinamide was measured in serum as described by Brodie, Levy, and Bernstein (15). Pre-injection samples of serum and urine were used for blank determinations in all clearances; the urine blank excretions were calculated as milligrams per minute. Endogenous creatinine was determined as described by R. S. Hare (3) except that 5 per cent trichloracetic acid filtrates of serum and urine were used, as has since been advocated by Mandel and Jones (16).

[1] Presented in part before the Society for Pediatric Research at Old Point Comfort, Virginia, May 5, 1952.

[2] This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

[3] Mead Johnson—Society for Pediatric Research Fellow in Pediatrics, 1951.

[4] Here and throughout this paper, the term "creatinine" refers to endogenous creatinine measured by specific methods.

[5] We are indebted to Dr. William Boger, of Sharp and Dohme, Inc., for supplying PAH and Carinamide and to Dr. Ernst Oppenheimer, of Ciba Pharmaceutical Products, Inc., for the DCG.

RESULTS

Creatinine: inulin clearance ratios in children with and without kidney disease

Individual values for the ratio of C_{CR}: C_{IN} in 12 observations on 11 children without kidney disease and in 105 observations on 57 children with kidney disease are shown in Figure 1. The mean value and standard deviation for the ratio in children without kidney disease were 1.03 ± 0.11. Although data from the children with kidney disease show a wide scatter, the ratio tended to be markedly elevated in those children who had reduced C_{IN}.

Thiosulfate: inulin clearance ratios in children with kidney disease

Values for simultaneously determined ratios of C_{THIO}: C_{IN} and C_{CR}: C_{IN} in 36 observations on 25 children with kidney disease are shown in Figure 2. Although the scatter of the data is again large, it is apparent that in contrast to the ratio of C_{CR}: C_{IN} the range of values for C_{THIO}: C_{IN} is the same at all levels of C_{IN} and centers about unity.

Despite the evidence that there may be slight tubular secretion and reabsorption of thiosulfate (17–19), these data suggest that C_{IN} and C_{THIO}, rather than C_{CR}, are at the level of GFR. If C_{CR} measured GFR in the children with reduced kidney function, one would have to conclude that the net effect of tubular transfer of thiosulfate and inulin was approximately the same; this is unlikely for two substances of such different physical-chemical properties.

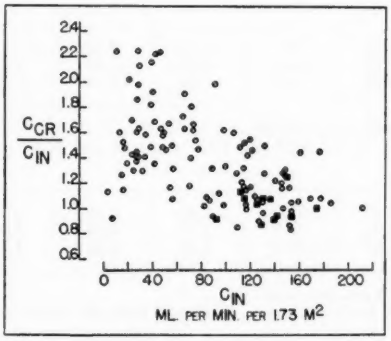

FIG. 1. CREATININE: INULIN CLEARANCE RATIOS (C_{CR}/C_{IN}) IN CHILDREN WITH (OPEN CIRCLES) AND WITHOUT (BLACK SQUARES) KIDNEY DISEASE PLOTTED AGAINST THE INULIN CLEARANCE (C_{IN})

Each symbol represents the integrated mean value for three consecutive clearance periods.

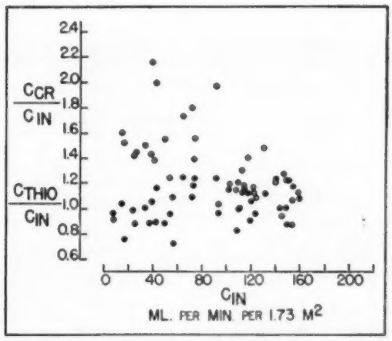

FIG. 2. SIMULTANEOUSLY DETERMINED CLEARANCE RATIOS OF CREATININE: INULIN (C_{CR}/C_{IN}, OPEN CIRCLES), AND THIOSULFATE:INULIN (C_{THIO}/C_{IN}, BLACK DOTS), IN CHILDREN WITH KIDNEY DISEASE PLOTTED AGAINST THE INULIN CLEARANCE, C_{IN}

Inulin clearances at various serum inulin concentrations in children with kidney disease

C_{IN} was measured at three widely separated ranges of serum inulin concentrations in each of two children (LA, SV) with and in one (OR) without kidney disease. Simultaneous measurements of C_{CR}, C_U, and C_{PAH} were made. The results are given in Table I. It can be seen that changes in serum inulin concentrations, varying from 16 to 207, from 12 to 61, and from 24 to 112 mgm. per 100 ml., had no consistent effect on C_{IN}, or on the ratios of C_{CR}: C_{IN}, C_U: C_{IN}, and C_{PAH}: C_{IN}.

Equations for the best fit lines relating serum inulin concentration, S_{IN}, to rate of inulin excretion, $U_{IN}V$, were calculated by the method of least squares from the data on LA, SV, and OR. These equations are given in Table IA and the lines for LA and SV are shown graphically in Figure 3 together with the observed values. The intercept of the curve on the $U_{IN}V$ axis (inulin excretion) does not differ statistically (Table IA) from the origin in either of the children with or in the child without kidney disease. Hence the data support the conclusion that the excretion of inu-

TABLE I

Effect of varying concentrations of inulin in serum on clearances of inulin (C_{IN}) and on ratios of clearances of creatinine (C_{CR}), urea (C_U) and para-aminohippurate (C_{PAH}) to C_{IN} in two children with and one without kidney disease

Subject	Elapsed time	Urine flow	Inulin			$\frac{C_{CR}}{C_{IN}}$	$\frac{C_U}{C_{IN}}$	$\frac{C_{PAH}}{C_{IN}}$‡
			Serum†	Excreted	Clearance			
	min.	*ml./min.*	*mgm./100 ml.*	*mgm./min.*	*ml./min.*			
LA	0–16	0.21						
Age: 3 yrs. Height: 95 cms. Weight: 19.9 kgm. S. A.: 0.60 M^2*	28–32	Priming infusion: 7 ml. of 10 per cent inulin and 1.2 ml. of 20 per cent PAH. Sustaining infusion: 0.53 gm. inulin, 0.27 gm. PAH and 0.90 gm. NaCl per 100 ml., given at 0.5 ml./min.						
Mean C_{IN} = 7.8 ml./min. = 18 per cent normal	75–88	0.21	17.0	1.31	7.7	1.65	0.70	3.81
	89–102	0.24	16.3	1.28	7.9	1.75	0.72	4.01
	103–115	0.25	15.7	1.29	8.2	1.68	0.79	4.16
	117–121	Priming infusion: 20 ml. of 10 per cent inulin. Sustaining infusion: 1.66 gm. inulin, 0.27 gm. PAH and 0.90 gm. NaCl per 100 ml., given at 0.5 ml./min.						
	155–168	0.21	63.0	4.88	7.8	1.72	0.68	4.15
	169–182	0.26	59.1	4.95	8.4	1.65	0.77	4.30
	183–196	0.28	55.5	4.52	8.1	1.79	0.84	4.60
	198–202	Priming infusion: 55 ml. of 10 per cent inulin. Sustaining infusion: 4.0 gm. inulin, 0.27 gm. PAH and 0.90 gm. NaCl per 100 ml., given at 0.5 ml./min.						
	241–254	0.43	206.5	15.81	7.7	1.79	0.84	4.61
	255–268	0.38	193.7	14.09	7.3	1.86	0.88	5.05
	269–282	0.34	183.2	13.02	7.1	2.01	1.00	5.10
SV	0–14	0.52						
Age: 3 yrs. Height: 98 cms. Weight: 19.9 kgm. S. A.: 0.64 M^2*	17–20	Priming infusion: 7 ml. of 10 per cent inulin and 1.5 ml. of 20 per cent PAH. Sustaining infusion: 0.40 gm. inulin, 0.20 gm. PAH and 0.90 gm. NaCl in 100 ml., given at 0.5 ml./min.						
Mean C_{IN} = 27 ml./min. = 59 per cent normal	50–67	0.46	16.6	4.34	26.1	1.39	0.58	7.49
	68–80	0.45	14.1	3.48	24.7	1.58	0.64	7.36
	81–93	0.48	12.1	3.01	24.9	1.53	0.64	7.46
	98–102	Priming infusion: 16 ml. of 10 per cent inulin. Sustaining infusion: 1.17 gm. inulin, 0.20 gm. PAH and 0.90 gm. NaCl in 100 ml., given at 0.5 ml./min.						
	132–143	0.46	35.8	9.65	27.0	1.35	0.48	6.06
	144–156	0.41	32.4	8.26	25.5	1.63	0.60	7.05
	157–168	0.42	29.5	8.89	30.1	1.30	0.44	5.50
	171–175	Priming infusion: 25 ml. of 10 per cent inulin. Sustaining infusion: 3.0 gm. inulin, 0.20 gm. PAH and 0.90 gm. NaCl in 100 ml., given at 0.5 ml./min.						
	216–227	0.45	61.1	18.85	30.9	1.49	0.44	6.47
	228–239	0.43	57.6	16.18	28.1	1.53	0.51	7.36
	240–251	0.42	54.3	13.43	24.7	1.60	0.58	7.62

TABLE I—*Continued*

Subject	Elapsed time	Urine flow	Inulin			$\frac{C_{CR}}{C_{IN}}$	$\frac{C_U}{C_{IN}}$	$\frac{C_{PAH}‡}{C_{IN}}$
			Serum†	Excreted	Clearance			
	min.	*ml./min.*	*mgm./100 ml.*	*mgm./min.*	*ml./min.*			
OR Age: 8 yrs. Height: 136.5 cms. Weight: 43 kgm. S. A.: 1.22 M²* Mean C_{IN} = 76.2 ml./min. = 76 per cent normal	0–10	6.15						
	14–18	Priming infusion: 14 ml. of 10 per cent inulin and 2.1 ml. of 20 per cent PAH. Sustaining infusion: 1.28 gm. inulin, 0.50 gm. PAH and 0.60 gm. NaCl per 100 ml., given at 1.5 ml./min.						
	52–61	1.54	26.1	19.35	74.1	1.23	0.47	5.61
	62–71	1.69	25.2	18.98	75.3	1.26	0.53	5.65
	72–81	2.58	24.2	18.53	76.6	1.28	0.57	5.81
	83–87	Priming infusion: 30 ml. of 10 per cent of inulin. Sustaining infusion: 4.25 gm. inulin, 0.50 gm. PAH and 0.60 gm. NaCl per 100 ml., given at 1.5 ml./min.						
	124–133	1.41	64.0	46.60	72.8	1.28	0.47	5.46
	134–143	1.35	62.1	44.60	71.8	1.25	0.52	5.50
	144–153	1.71	60.3	47.00	77.9	1.16	0.49	5.44
	155–159	Priming infusion: 43 ml. of 10 per cent inulin. Sustaining infusion: 8.50 gm. inulin, 0.50 gm. PAH and 0.60 gm. NaCl per 100 ml., given at 1.5 ml./min.						
	194–203	1.40	112.0	83.50	74.8	1.16	0.49	4.79
	204–213	1.70	106.4	87.50	82.7	1.18	0.55	5.09
	214–223	1.62	101.3	80.00	79.2	1.18	0.51	4.75

* Ideal weight for height and age (21) was used for the estimation of surface area (22) from which expected normal values for renal clearances were calculated.
† Values interpolated to mid-point of period.
‡ Serum PAH concentrations in mgm. per 100 ml. ranged from 2.92 to 1.85 in LA, from 1.34 to 0.42 in SV and from 1.05 to 1.78 in OR.

lin is linearly proportional to the concentration of inulin in serum.

These results, which confirm earlier observations of Miller and Winkler (8), further suggest that C_{IN} measures GFR in these children. If there were tubular reabsorption of inulin, and if there were a limiting rate of reabsorption within the range of serum inulin concentrations obtained, one

TABLE IA

Statistical summary

Subject	Equation of best fit line*	Standard deviation of intercept†	t‡	P§
LA	$U_{IN}V = 0.251 + 0.073\ S_{IN}$	±0.189	1.325	0.22
SV	$U_{IN}V = -0.606 + 0.292\ S_{IN}$	±0.702	0.863	0.41
OR	$U_{IN}V = -1.68 + 0.793\ S_{IN}$	±2.35	0.716	0.50

* Calculated by method of least squares. $U_{IN}V$ is given in mgm. per minute and S_{IN} in mgm. per 100 ml.
† Calculated as described by Youden (20).
‡ t = Intercept ÷ standard deviation of intercept.
§ P is the probability that the observed intercept deviates from the origin by chance alone.

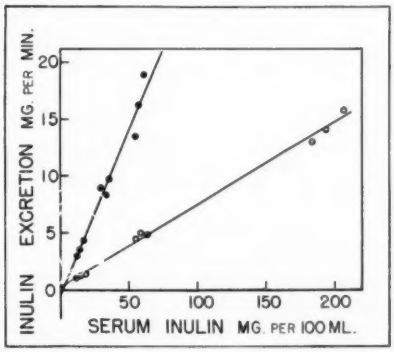

FIG. 3. RELATIONSHIP BETWEEN CONCENTRATION OF INULIN IN SERUM AND RATE OF INULIN EXCRETION IN TWO CHILDREN WITH KIDNEY DISEASE

See text for explanation of fitted lines.

TABLE II

Clearances of inulin (C_{IN}), para-aminohippurate (C_{PAH}), creatinine (C_{CR}) and urea (C_U) and ratios of C_{PAH}:C_{IN} and C_{CR}:C_{IN} before and after single and repeated doses of Carinamide in four children with the nephrotic syndrome

Subject	Age	Surface area	Date	C_{IN}	C_{PAH}	C_{CR}	C_U	$\frac{C_{PAH}}{C_{IN}}$	$\frac{C_{CR}}{C_{IN}}$	Serum Carinamide*
	yrs.	*M²*		*ml./min./1.73 M²*						*mgm./100 ml.*
BE	5	0.75	Nov. 27, 1951	55.0	269	79.8	30.4	4.90	1.45	0
				Carinamide: 0.13 gm./Kgm. 1 dose						
				47.4†	117	66.7	28.7	2.47	1.41	5.6–7.7
LO	4	0.66	Nov. 7, 1951	28.6	97	40.1	20.1	3.39	1.40	0
			Nov. 8 to 9, 1951	Carinamide: 0.04 gm./Kgm. q 4h 7 doses						
			Nov. 9. 1951	24.0†	28	34.3	17.0	1.17	1.43	3.6–6.4
			Jan. 21, 1952	26.5	80	36.7	18.6	3.02	1.38	0
				Carinamide: 0.36 gm./Kgm. 1 dose						
				23.8†	28	32.0	16.0	1.18	1.34	4.7–13.3
			Feb. 11 to 13, 1952	Carinamide: 0.06 gm./Kgm. q 6h 9 doses						
			Feb. 13, 1952	22.8†	23	25.9	15.9	1.01	1.14	5.7–6.9
KR	8	0.95	Dec. 28, 1951	25.8	84	37.3	22.7	3.26	1.45	0
			Dec. 31, 1951 to Jan. 2, 1952	Carinamide: 0.04 gm./Kgm. q 4h 13 doses						
			Jan. 2, 1952	22.0†	25	26.0	19.7	1.14	1.18	21.4–25.1
RI	2½	0.54	June 8, 1951	49.3	398	79.0	—	8.07	1.60	0
			June 12 to 15, 1951	Carinamide: 0.03 gm./Kgm. q 3h 24 doses						
			June 15, 1951	41.3†	53	47.4	—	1.28	1.15	?

* Concentrations at beginning and end of clearance periods.

† Post-Carinamide clearances were started between 1.5 and 2 hours after either a single dose or after the last of the repeated doses.

would expect, as pointed out by Shannon and Smith (23), that the amount excreted per unit time would not have been related to serum inulin concentrations in the manner shown in Figure 3. Rather, the curve would, when extrapolated, intersect the ordinate at a negative value.

Contrary to these and the earlier results mentioned (8, 23), Ferguson and his associates (24) have recently reported observations in which lines similarly plotted did intersect the ordinate at a negative value, from which they conclude that tubular reabsorption of inulin occurs. However, in their four observations in three subjects to whom continuous intravenous infusions of inulin were given, the inulin was dissolved in physiologic saline and different serum inulin concentrations

were achieved by changing the rate of infusion rather than by changing the concentration of inulin in the infusion fluid as done here. In the interpretation of all these observations, it is assumed that GFR remains constant throughout. Since each of the 3 subjects studied by Ferguson and his co-workers (24) had hypertension and one was 70 years old, it is possible that changes in the rate of infusion of saline induced changes in GFR. Such induced changes in GFR, if they occurred, could explain the contradictory results.

Effect of Carinamide on the creatinine: inulin clearance ratio in children with kidney disease

If, as suggested by the observations above, C_{IN} measures GFR in children with kidney disease, it follows that creatinine is secreted by the tubules and that it may be possible to suppress this function.[6] Carinamide is known to suppress the tubular secretion of a number of substances (26–28). The effect of varying amounts of Carinamide given by mouth to four children with kidney disease (BE, LO, KR, RI) and with high ratios of C_{CR}: C_{IN} is shown in Table II. It can be seen that a single dose of 0.13 gm. per kgm. given to LO on January 21, 1952 failed to suppress the ratio of C_{CR}: C_{IN}. Similarly, a dosage of 0.04 gm. per kgm. given to LO at four-hour intervals for seven doses had no effect on the ratio measured one hour after the last previous dose on November 9, 1951. However, doses of 0.03 to 0.06 gm. per kgm. given at three- to six-hour intervals for nine to 24 doses decreased the ratio toward one in LO, KR, and RI. The constancy of the ratio, as well as of C_{IN}, in the four observations on LO over a period of three months strongly suggests that the decrease after nine doses of Carinamide was, in fact, due to the drug.

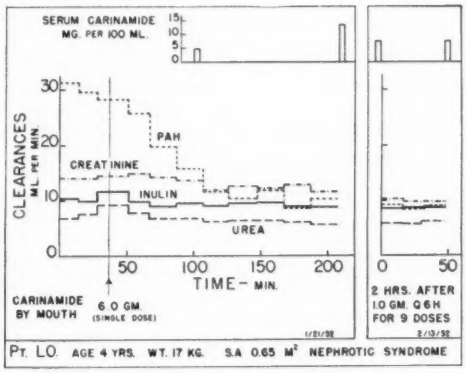

FIG. 4. CLEARANCES OF PARA-AMINOHIPPURATE (PAH), CREATININE, INULIN, AND UREA IN A CHILD WITH KIDNEY DISEASE BEFORE AND AFTER A SINGLE DOSE AND AFTER REPEATED DOSES OF CARINAMIDE BY MOUTH

From these observations, which confirm the earlier reports of Brod and Sirota (29) and Brod and Kotatko (25), it would appear that the high ratio of C_{CR}: C_{IN} occurring in children with kidney disease and reduced C_{IN} can be brought toward one by a decrease in C_{CR} with no marked change in C_{IN}. It is of interest as shown in Figure 4 that when Carinamide had been given to LO every six hours for nine doses and when creatinine secretion was suppressed the concentration of Carinamide in serum was actually lower than that observed after a single large dose which failed to suppress secretion of creatinine. It can also be seen in Figure 4 that C_{PAH} was reduced to the level of C_{IN} and below the simultaneously measured C_{CR}. This observation strongly supports the conclusion that C_{IN} rather than C_{CR} measures GFR. If C_{CR} were at the level of GFR, one would have to conclude that Carinamide not only suppresses tubular secretion but causes tubular reabsorption of PAH which is unlikely.[7]

[6] Brod and Kotatko (25) have reported that in patients with kidney disease who had high ratios of C_{CR}: C_{IN}, high concentrations of PAH in serum depressed the ratio toward one. However, in 5 observations on children with kidney disease who had high ratios of C_{CR}: C_{IN} (1.6 to 2.3) and in whom the ratio was measured at low (0.65 to 1.52 mgm. per 100 ml.) and high (56 to 88 mgm. per 100 ml.) serum PAH concentrations, we observed an *apparent increase* (4 to 32%) in the ratio in four and no apparent change in the fifth. We have found that high concentrations of PAH (25 mgm. per 100 ml.) in a creatinine solution adsorbed onto Lloyd's reagent cause an increase in the color obtained in the Jaffe reaction done on the eluate. Although the magnitude of this effect could account for the apparent *increases* in the ratio of C_{CR}: C_{IN}, we do not believe it is great enough to conceal significant *decreases* in the ratio. We cannot explain the discrepancy between these results and those reported by Brod and Kotatko (25).

[7] Further support would be given to this conclusion if complete suppression of glucose reabsorption raised the clearance of glucose (C_G) to the level of C_{IN} rather than to C_{CR}. Suppression of glucose reabsorption was at-

COMMENT

The data presented, if extended and confirmed, would appear to validate C_{IN} as a measure of GFR in children with kidney disease. However, as is true in normal subjects, the evidence for this concept is indirect. The evidence rests in part on the demonstration that C_{IN} is independent of changes in serum inulin concentration within wide limits, which appears to be true in the present data on children with kidney disease. However, in both the normal and diseased kidney, it is possible, though unlikely, that a very small but constant percentage of filtered inulin is reabsorbed. This type of tubular reabsorption with no definite upper limiting rate is generally believed to occur with urea.

In addition, there is a greater possibility in patients with kidney disease than in the normal subject that there could be more or less complete reabsorption of glomerular filtrate in some nephrons. If so, as stated by Smith (1), it remains beyond the possibility of examination as long as only the total urine is available for analysis. However, even if complete local reabsorption occurs, C_{IN} would still measure the rate of filtration of that part of the glomerular fluid which is elaborated into urine.

These criticisms of part of the evidence supporting the validity of C_{IN} as a measure of GFR do not alter the inferences drawn from the observations with Carinamide concerning tubular secretion of creatinine in children with kidney disease.

The data presented have certain clinical as well as physiologic significance. It has been suggested by Brod and Sirota (27) that "creatinine" chromogen (total creatinine chromogen) clearances may provide a useful clinical approximation of GFR in subjects with kidney disease. On the other hand, Miller and his associates (7) conclude that neither total chromogen nor specific endogenous creatinine clearances should be employed as a precise measure of GFR in patients with kidney disease. Our data strongly suggest that C_{CR} provides neither a precise nor an approximate measure of GFR in children with kidney disease.

If one or two large doses of Carinamide had consistently depressed the ratio of $C_{CR}:C_{IN}$ to unity in children with kidney disease, it might have been possible to utilize this effect in devising a relatively simple means of measuring GFR in these children. However, since Carinamide had to be given over a period of at least 2 days to depress the ratio, it does not appear clinically feasible to use this drug for this purpose.

tempted with desoxycorticosterone glucoside (30, 31). However, 100 mgm. given in a single injection intravenously raised C_0 only to 60 per cent of C_{IN} in an 8-year-old child without kidney disease. The same dose in two children, 2 and 3 years old, with kidney disease and reduced C_{IN} increased C_0 only to 18 and 40 per cent of C_{IN}. The smaller effect in children with reduced function dissuaded us from using larger doses to try to get complete inhibition of glucose reabsorption.

SUMMARY AND CONCLUSIONS

The data presented indicate that, using a specific method for measuring endogenous creatinine in serum and urine, the creatinine: inulin clearance ratio is close to unity in normal children, but tends to be markedly elevated in children with kidney disease and reduced kidney function.

In observations designed to investigate whether the clearance of inulin or of creatinine provides a valid measure of glomerular filtration rate in children with kidney disease and with high creatinine: inulin clearance ratios, it has been found that in these children:

(a) thiosulfate: inulin clearance ratios are close to unity;

(b) the inulin clearance is constant over a wide range of concentrations of inulin in serum;

(c) Carinamide in dosages that fail to lower the creatinine: inulin clearance ratio depresses the clearance of *p*-aminohippurate to the level of the inulin clearance and below the simultaneously measured creatinine clearances; and

(d) prolonged oral administration of Carinamide may decrease the creatinine: inulin ratio toward one by decreasing the creatinine clearance without increasing the inulin clearance.

The data suggest that:

(a) the clearance of inulin does provide a valid measure of glomerular filtration rate in children with kidney disease;

(b) in children with high creatinine: inulin clearance ratios, creatinine is secreted by the tubules; and

(c) using specific creatinine methods, the endogenous creatinine clearance may measure glomerular filtration rate in normal children, but that creatinine clearances *cannot* be used to measure glomerular filtration rate in children with kidney disease.

ACKNOWLEDGMENTS

We are indebted to Flora Hurwitz, R.N. for nursing and technical assistance and to Emily Matejka and Gladys Folkart for technical assistance.

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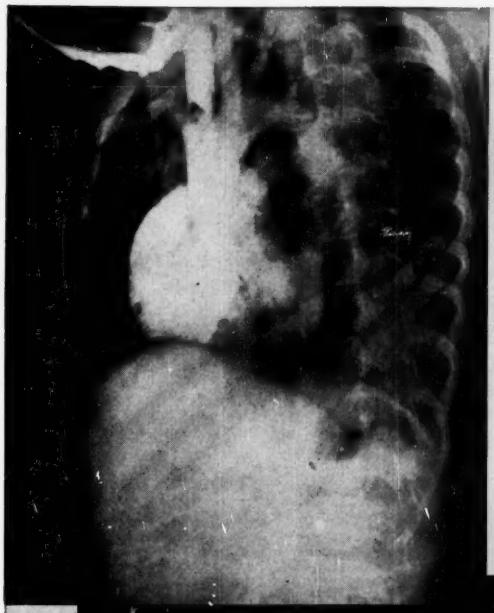

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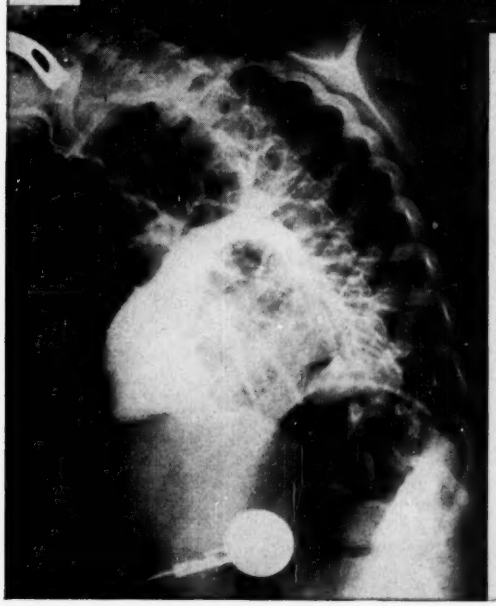

Specific instances in which it may be of unsurpassed diagnostic value are congenital lesions such as patent ductus arteriosus, tetralogy of Fallot, coarctation of the aorta, patent foramen ovale, dextrocardia, etc.; aneurysms, mediastinal lesions and chronic pericarditis.

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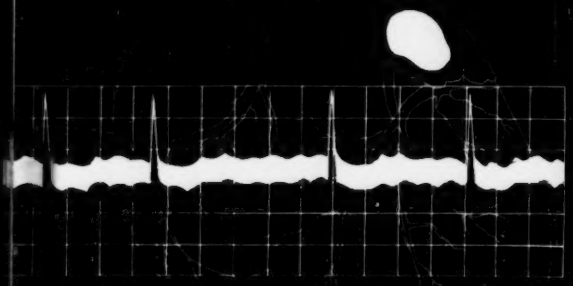

in congestive heart failure

Accepted therapeutic measures in congestive heart failure, useful though they are, may themselves aggravate the already existing predisposition to thrombo-embolic complication.

While prophylactic heparinization may not be indicated in every case, it is particularly worth considering when the clinical picture includes regional stasis, auricular fibrillation, marked atherosclerosis or previous thrombo-embolism.

Depo-Heparin is easily administered and provides prolonged control of thrombo-embolism.

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Each cc. contains:	*Heparin Sodium*	*20,000 U.S.P. units (approx. 200 mg.)*
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	Dextrose, Anhydrous	*80 mg.*
	Water for Injection	*q.s.*

Preserved with sodium ethyl mercuri thiosalicylate 1:10,000.

Supplied with sterile disposable 1 cc. cartridge syringe.

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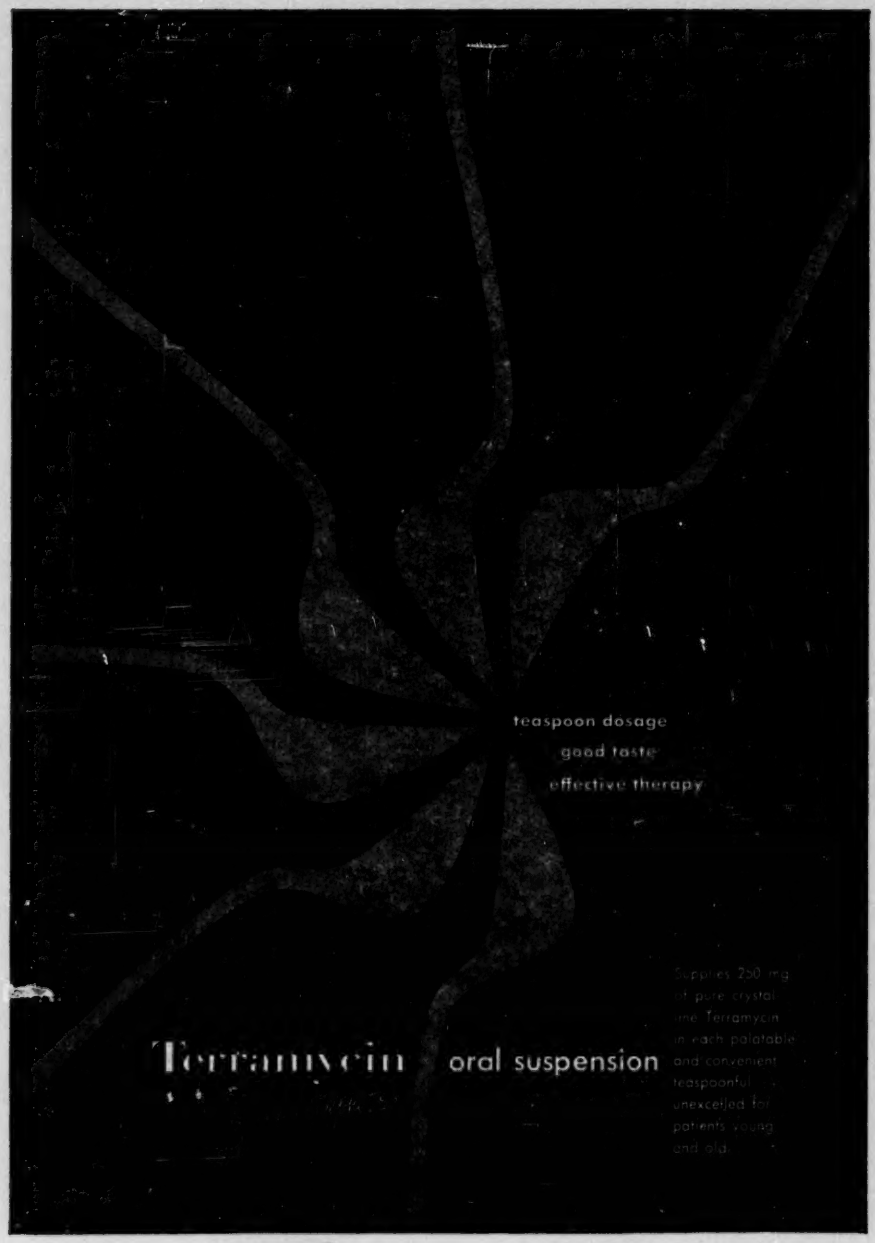
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